PB81-124414

Organic Analyses in Water Quality Control Programs - Training Manual

(U.S.) National Training and Operational
Technology Center, Cincinnati, OH

Nov 80

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

National Training
and Operational
Technology Center
Cincinnati OH 45268

EPA-430/1-80-011
September 1980

PB81-124414

Water

EPA

Organic Analyses in Water Quality Control Programs

Training Manual

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-430/1/80-011

2.

3. RECIPIENT'S ACCESSION NO.
PB81 124414

4. TITLE AND SUBTITLE
ORGANIC ANALYSES IN WATER QUALITY CONTROL PROGRAMS - TRAINING MANUAL

5. REPORT DATE
November 1980

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Charles Feldmann

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
National Training & Operational Technology Center
26 West St. Clair Street
Cincinnati, Ohio 45268

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
N/A

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES
Supercedes PB297-713/AS

16. ABSTRACT

A lecture/laboratory manual dealing with the analysis of selected organic pollutants. Intended for use by those having little or no experience in the field, but having one year (or equivalent) of college organic chemistry, and having basic laboratory skills (volumetric glassware, titration assemblies, analytical and trip balances). Topics include dissolved oxygen, biochemical oxygen demand, ammonia, nitrates, nitrites, carbon analysis chemical oxygen demand, surfactants, oil and grease phenolics, gas chromatography, and polychlorinated biphenyls.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chemical analysis, analysis, instrumentation, pollutant identification, water analysis	N/A	07/C

18. DISTRIBUTION STATEMENT Released unlimited	**19. SECURITY CLASS** *(This Report)* None	**21. NO. OF PAGES**
	20. SECURITY CLASS *(This page)* None	**22. PRICE**

EPA Form 2220-1 (9-73)

EPA-430/1-80-011
September 1980

Organic Analyses in Water Quality Control Programs

This course is for chemists and technicians with little or no experience in organic analyses commonly required for NPDES and NIPDWR regulations and for other water quality programs. Applicants should have a fundamental knowledge of organic chemistry and quantitative analyses, and should have basic laboratory skills including the use of volumetric glassware and titration assemblies. They should be actively engaged in a water quality control program.

After successfully completing the course, the student will know how to perform analyses for selected organic pollutants listed as approved in the Federal Register.

The training is a five-day course which includes classroom instruction, student performance of laboratory procedures, and discussion of each laboratory assignment and reported results.

Course topics include NPDES methodology, laboratory safety, dissolved oxygen (Winkler), five-day biochemical oxygen demand, total organic carbon, oil and grease (separatory funnel extraction), surfactants (MBAS), total Kjeldahl and organic nitrogen (Nesslerization), phenol (4-AAP), polychlorinated biphenyls, pesticides, and control of analytical performance. Emphasis is on laboratory operations.

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
National Training and Operational Technology Center

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Program Operations, U. S. Environmental Protection Agency.

> Due to the limited availability of the manuals it is not appropriate to cite them as technical references in bibliographies or other forms of publication.
>
> References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Program Operations, U. S. Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

CONTENTS

Title or Description	Outline Number
Laboratory Safety Practices	1
Sample Handling - Field Through Laboratory	2
Methodology For Chemical Analysis Of Water And Wastewater	3
Statistics For Chemists	4
Accuracy-Precision-Error	5
Elements Of A Quality Assurance Program	6
Use Of A Spectrophotometer	7
Dissolved Oxygen - Factors Affecting DO Concentration In Water	8
Dissolved Oxygen - Determination by the Winkler Iodometric Titration - Azide Modification	9
Laboratory Procedure For Dissolved Oxygen Winkler Method - Azide Modification	10
Dissolved Oxygen-Determination By Electronic Measurement	11
Biochemical Oxygen Demand Test Procedures	12
Biochemical Oxygen Demand Test Dilution Technique	13
Sources And Analysis Of Organic Nitrogen	14
Ammonia, Nitrites And Nitrates	15
Determination Of Kjeldahl Nitrogen (Micro Apparatus-Nesslerization)	16
Total Carbon Analysis	17
Chemical Oxygen Demand And COD/BOD Relationships	18
Laboratory For Routine Level Chemical Oxygen Demand Determination	19
Determination Of Surfactants	20
Laboratory Determination Of Surfactants (Methylene Blue Active Substances, MBAS)	21
Oil And Grease	22
Laboratory Determination Of Oil And Grease	23
Determination Of Phenolics	24
Laboratory Determination Of Phenol Direct Photometric Method	25
Introduction To Gas-Liquid Chromatography	26
Quantitation In Gas Chromatography	27
Polychlorinated Biphenyls	28
Method For Polychlorinated Biphenyls (PCB's) In Industrial Effluents	29
Method For Organochlorine Pesticides In Industrial Effluents	30

LABORATORY SAFETY PRACTICES

I INTRODUCTION

A Safe Use, Handling and Storage of Chemicals

1 Chemicals in any form can be safely stored, handled, and used if their hazardous physical and chemical properties are fully understood and the necessary precautions, including the use of proper safeguards and personal protective equipment are observed.

2 The management of every unit within a manufacturing establishment must give wholehearted support to a well integrated safety policy.

B General Rules for Laboratory Safety

1 Supervisory personnel should think "safety." Their attitude toward fire and safety standard practices is reflected in the behavior of their entire staff.

2 A safety program is only as strong as the worker's will to do the correct things at the right time.

3 The fundamental weakness of most safety programs lies in too much lip service to safety rules and not enough action in putting them into practice.

4 Safety practices should be practical and enforceable.

5 Accident prevention is based on certain common standards of education, training of personnel and provision of safeguards against accidents.

II LABORATORY DESIGN AND EQUIPMENT

A Type of Construction

1 Fire-resistant or noncombustible

2 Multiple story buildings should have adequate means of exit.

3 Stairways enclosed with brick or concrete walls

4 Laboratories should have adequate exit doors to permit quick, safe escape in an emergency and to protect the occupants from fires or accidents in adjoining rooms. Each room should be checked to make sure there is no chance of a person being trapped by fire, explosions, or release of dangerous gases.

5 Laboratory rooms in which most of the work is carried out with flammable liquids or gases should be provided with explosion-venting windows.

B Arrangement of Furniture and Equipment

1 Furniture should be arranged for maximum utilization of available space and should provide working conditions that are efficient and safe.

2 Aisles between benches should be at least 4 feet wide to provide adequate room for passage of personnel and equipment.

3 Desks should be isolated from benches or adequately protected.

4 Every laboratory should have an eye-wash station and a safety shower.

C Hoods and Ventilation

1 Adequate hood facilities should be installed where work with highly toxic or highly flammable materials are used.

2 Hoods should be ventilated separately and the exhaust should be terminated at a safe distance from the building.

3 Make-up air should be supplied to rooms or to hoods to replace the quantity of air exhausted through the hoods.

4 Hood ventilation systems are best designed to have an air flow of not less than 60 linear feet per minute across the face of the hood, with all doors open and 150, if toxic materials are involved.

5 Exhaust fans should be spark-proof if exhausting flammable vapors and corrosive resistant if handling corrosive fumes.

6 Controls for all services should be located at the front of the hood and should be operable when the hood door is closed.

7 All laboratory rooms should have the air changed continuously at a rate depending on the materials being handled.

D Electrical Services

1 Electrical outlets should be placed outside of hoods to afford easy access and thus protect them from spills and corrosion by gases.

2 Noninterchangeable plugs should be provided for multiple electrical services.

3 Adequate outlets should be provided and should be of the three-pole type to provide for adequate grounding.

E Storage

1 Laboratories should provide for adequate storage space for mechanical equipment and glassware which will be used regularly.

2 Flammable solvents should not be stored in glass bottles over one liter in size. Large quantities should be stored in metal safety cans. Quantities requiring containers larger than one gallon should be stored outside the laboratory.

3 Explosion proof refrigerators should be used for the storage of highly volatile and flammable solvents.

4 Cylinders of compressed or liquified gases should not be stored in the laboratory.

F Housekeeping

1 Housekeeping plays an important role in reducing the frequency of laboratory accidents. Rooms should be kept in a neat orderly condition. Floors, shelves, and tables should be kept free from dirt and from all apparatus and chemicals not in use.

2 A cluttered laboratory is a dangerous place to work. Maintenance of a clean and orderly work space is indicative of interest, personal pride, and safety-mindedness.

3 Passageways should be kept clear to all building exits and stairways.

4 Metal containers should be provided for the disposal of broken glassware and should be properly labeled.

5 Separate approved waste disposal cans, should be provided for the disposal of waste chemicals.

6 Flammable liquids not miscible with water and corrosive materials, or compounds which are likely to give off toxic vapors should never be poured into the sink.

G Fire Protection

1 Laboratory personnel should be adequately trained regarding pertinent fire hazards associated with their work.

2 Personnel should know rules of fire prevention and methods of combating fires.

3 Fire extinguishers (CO_2 type) should be provided at convenient locations and personnel should be instructed in their use.

4 Automatic sprinkler systems are effective for the control of fires in chemical laboratories.

H Alarms

1 An approved fire alarm system should be provided.

2 Wherever a hazard of accidental release of toxic gases exists, a gas alarm system to warn occupants to evacuate the building should be provided.

3 Gas masks of oxygen or compressed air type should be located near exits and selected personnel trained to use them.

III HANDLING GLASSWARE

A Receiving, Inspection and Storage

1 Packages containing glassware should be opened and inspected for cracked or nicked pieces, pieces with flaws that may become cracked in use, and badly shaped pieces.

2 Glassware should be stored on well-lighted stockroom shelves designed and having a coping of sufficient height around the edges to prevent the pieces from falling off.

B Laboratory Practice

1 Select glassware that is designed for the type of work planned.

2 To cut glass tubing or a rod, make a straight clean cut with a cutter or file at the point where the piece is to be severed. Place a towel over the piece to protect the hands and fingers, then break away from the body.

3 Large size tubing is cut by means of a heated nichrome wire looped around the piece at the point of severance.

4 When it is necessary to insert a piece of glass tubing or a rod through a perforated rubber or cork stopper, select the correct bore so that the insertion can be made without excessive strain.

5 Use electric mantels for heating distillation apparatus, etc.

6 To remove glass splinters, use a whisk broom and a dustpan. Very small pieces can be picked up with a large piece of wet cotton.

IV GASES AND FLAMMABLE SOLVENTS

A Gas Cylinders

1 Large cylinders must be securely fastened so that they cannot be dislodged or tipped in any direction.

2 Connections, gauges, regulators or fittings used with other cylinders must not be interchanged with oxygen cylinder fittings because of the possibility of fire or explosion from a reaction between oxygen and residual oil in the fitting.

3 Return empty cylinders promptly with protective caps replaced.

B Flammable Solvents

1 Store in designated areas well ventilated.

2 Flash point of a liquid is the temperature at which it gives off vapor sufficient to form an ignitible mixture with the air near the surface of the liquid or within the vessel used.

3 Ignition temperature of a substance is the minimum temperature required to initiate or cause self-sustained combustion independently of the heating or heated element.

4 Explosive or flammable limits. For most flammable liquids, gases and solids there is a minimum concentration of vapor in air or oxygen below which propagation of flame does not occur on contact with a source of ignition. There is also a maximum proportion of vapor or gas in air above which

propagation of flame does not occur. These limit mixtures of vapor or gas with air, which if ignited will just propagate flame, are known as the "lower and higher explosive or flammable limits."

5 Explosive Range. The difference between the lower and higher explosive or flammable limits, expressed in terms of percentage of vapor or gas in air by volume is known as the "explosive range."

6 Vapor Density is the relative density of the vapor as compared with air.

7 Underwriter's Laboratories Classification is a standard classification for grading the relative hazard of the various flammable liquids. This classification is based on the following scale:

Ether Class	100
Gasoline Class.........	90 - 100
Alcohol (ethyl) Class....	60 - 70
Kerosene Class	30 - 40
Paraffin Oil Class	10 - 20

8 Extinguishing agents

V CHEMICAL HAZARDS

A Acids and Alkalies

1 Some of the most hazardous chemicals are the "strong" or "mineral" acids such as hydrochloric, hydrofluoric, sulfuric and nitric.

2 Organic acids are less hazardous because of their comparatively low ionization potentials. However, such acids as phenol (carbolic acid), hydrocyanic and oxalic are extremely hazardous because of their toxic properties.

3 Classification of acids

B Oxidizing Materials

1 Such oxidizing agents as chlorates, peroxides, perchlorates and perchloric acid, in contact with organic matter can cause explosions and fire.

2 They are exothermic and decompose rapidly, liberating oxygen which reacts with organic compounds.

3 Typical hazardous oxidizing agents are:

Chlorine Dioxide
Sodium Chlorate
Potassium Chromate
Chromium Trioxide
Perchloric Acid

C Explosive Power

1 Many chemicals are explosive or form compounds that are explosive and should be treated accordingly.

2 A few of the more common examples of this class of hazardous materials are:

Acetylides
Silver Fulminate
Peroxides
Peracetic Acid
Nitroglycerine
Picric Acid
Chlorine and Ethylene
Sodium Metal
Calcium Carbide

D Toxicity

1 Laboratory chemicals improperly stored or handled can cause injury to personnel by virtue of their toxicity.

2 Types of exposure. There are four types of exposure to chemicals:

a Contact with the skin and eyes

b Inhalation

c Swallowing

d Injection

VI PRECAUTIONARY MEASURES

A Clothing and Personal Protective Equipment

1 Chemical laboratories should have special protective clothing and equipment readily available for emergency use and for secondary protection of personnel working with hazardous materials.

2 Equipment should be provided for adequate:

a Eye protection

b Body protection

c Respiratory protection

d Foot protection

e Hand protection

B Bodily Injury

1 Burns, eye injuries, and poisoning are the injuries with which laboratory people must be most concerned.

2 First emphasis in the laboratory should be on preventing accidents. This means observing all recognized safe practices using necessary personal protective equipment and exercising proper control over poisonous substances at the source of exposure.

3 So that a physician can be summoned promptly, every laboratory should have posted the names, telephone numbers, and addresses of doctors to be called in an emergency requiring medical care.

REFERENCES

Guide for Safety in the Chemical Laboratory, the General Safety Committee of the Manufacturing Chemists Association, Inc., Van Nostrand, New York (1954).

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Descriptors: Safety, Laboratory, Practices Safety, Laboratory Design Chemical Storage, Gas Cylinders, Flammable Solvents

SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

  a grab
  b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider. ASTM(1) has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met. (4)

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual(2) includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references (1, 2, 3) as part of the presentation of the individual procedures.

D Federal Register Methods

When collecting samples to be analyzed for National Pollutant Discharge Elimination System or State Certification report purposes, one must consult the appropriate Federal Register(5) for information about sample handling procedures. When collecting samples to be analyzed for compliance with maximum contaminant levels in drinking water, consult the EPA Report(6) which includes this information.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Environmental Monitoring and Support Laboratory of the Environmental Protection Agency, has published a manual of analytical procedures to provide methodology for monitoring the quality of our Nation's Waters and to determine the impact of waste discharges. The title of this manual is "Methods for Chemical Analysis of Water and Wastes."(2)

For some tests, this manual refers the analyst to Standard Methods and/or to ASTM for the stepwise procedure.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater."(3)

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 47 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards", Part 31, Water.(1)

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

E Federal Register Methodology

The analyst must consult the appropriate Federal Register for a listing of approved methodology if he is gathering data for National Pollutant Discharge Elimination System(5) or State Certification(5) report purposes, or to document compliance with maximum contaminant levels in drinking water(7). The Federal Register directs the user to pages in the above cited reference books where acceptable procedures can be found. The Federal Register also provides information concerning the protocol for obtaining approval to use analytical procedures other than those listed.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 24 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.(2)

3 The EPA Methods Manual(2) and Standard Methods(3) include a table summarizing holding times and preservation techniques for several analytical procedures. Additional information can be found in the standard references(1, 2, 3) as part of the presentation of the individual procedures.

4 A table with proposed holding times and preservation techniques applicable to samples collected for National Pollutant Discharge Elimination System or State Certification purposes was published in the December 18, 1979 Register(5). A similar table for drinking water samples can be found in a May, 1978 EPA Report(6).

5 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Information that should be recorded includes:

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used and why this was done
6 data obtained during analysis
7 results of quality control checks on the analysis
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using approved methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

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1 ASTM Annual Book of Standards, Part 31, Water, 1975.

2 Methods for Chemical Analysis of Water and Wastes, EPA-EMSL Cincinnati, Ohio 45268, 1979.

3 Standard Methods for the Examination of Water and Wastewater, 14th edition APHA-AWWA-WPCF, 1975.

4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

5 Federal Register, "Guidelines Establishing Test Procedures for the Analysis of Pollutants," Vol. 41, No. 232, December 1, 1976, pp pp 52780-52786. Also, Vol. 44, No. 244, December 18, 1979 pp 75028-75052 presents proposed changes. The latter is scheduled for finalization after January, 1981.

6 "Manual for the Interim Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies - Criteria and Procedures," U.S. EPA Report No. 600/8-78-008, May, 1978.

7 Federal Register, "National Interim Primary Drinking Water Regulations," Vol. 40, No. 248, December 24, 1975, pp 59566-59574. Also, 'Interim Primary Drinking Water Regulations; Amendments," Vol. 45, No. 168, August 27, 1980, pp 57332-57346.

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Descriptors: On-Site Data Collections, On-Site Investigations, Planning, Handling, Sample, Sampling, Water Sampling, Surface Waters, Preservation, Wastewater

METHODOLOGY FOR CHEMICAL ANALYSIS OF WATER AND WASTEWATER

I INTRODUCTION

This outline deals with chemical methods which are commonly performed in water quality laboratories. Although a large number of constituents or properties may be of interest to the analyst, many of the methods employed to measure them are based on the same analytical principles. The purpose of this outline is to acquaint you with the principles involved in commonly-used chemical methods to determine water quality.

II PRE-TREATMENTS

For some parameters, a preliminary treatment is required before the analysis begins. These treatments serve various purposes.

A Distillation - To isolate the constituent by heating a portion of the sample mixture to separate the more volatile part(s), and then cooling and condensing the resulting vapor(s) to recover the volatilized portion.

B Extraction - To isolate/concentrate the constituent by shaking a portion of the sample mixture with an immiscible solvent in which the constituent is much more soluble.

C Filtration - To separate undissolved matter from a sample mixture by passing a portion of it through a filter of specified size. Particles that are dissolved in the original mixture are so small that they stay in the sample solution and pass through the filter.

D Digestion - To change constituents to a form amenable to the specified test by heating a portion of the sample mixture with chemicals.

III METERS

For some parameters, meters have been designed to measure that specific constituent or property.

A pH Meters

pH (hydrogen ion concentration) is measured as a difference in potential across a glass membrane which is in contact with the sample and with a reference solution. The sensor apparatus might be combined into one probe or else it is divided into an indicating electrode (for the sample) and a reference electrode (for the reference solution). Before using, the meter must be calibrated with a solution of known pH (a buffer) and then checked for proper operation with a buffer of a different pH value.

B Dissolved Oxygen Meters

Dissolved oxygen meters measure the production of a current which is proportional to the amount of oxygen gas reduced at a cathode in the apparatus. The oxygen gas enters the electrode through a membrane, and an electrolyte solution or gel acts as a transfer and reaction media. Prior to use the meter must be calibrated against a known oxygen gas concentration.

C Conductivity Meters

Specific conductance is measured with a meter containing a Wheatstone bridge which measures the resistance of the sample solution to the transmission of an electric current. The meter and cell are calibrated according to the conductance of a standard solution of potassium chloride measured at 25°C by a "standard" cell with electrodes one cm square spaced one cm apart. This is why results are called "specific" conductance.

D Turbidimeters

A turbidimeter compares the intensity of light scattered by particles in the sample under defined conditions with the intensity of light scattered by a standard reference suspension.

IV SPECIFIC ION ELECTRODES

Just as the conventional glass electrode for pH develops an electrical potential in response to the activity of hydrogen ion in solution, the specific ion electrode develops an electrical potential in response to the activity of the ion for which the electrode is specific. The potential and activity are related according to the Nernst equation. Simple analytical techniques can be applied to convert activity to an expression of concentration.

These electrodes are used with a pH meter with an expanded mV scale or with a specific ion meter. Two examples are the ammonia and fluoride electrodes.

A Ammonia

The ammonia electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an ammonium chloride internal solution. Ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.

B Fluoride

The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The cell may be represented by Ag/Ag Cl, $Cl^-(0.3)$, $F^-(0.001)$ LaF/test solution/SCE/. It is used in conjunction with a standard single junction reference electrode.

V GENERAL ANALYTICAL METHODS

A Volumetric Analysis

Titrations involve using a buret to measure the volume of a standard solution of a substance required to completely react with the constituent of interest in a measured volume of sample. One can then calculate the original concentration of the constituent of interest.

There are various ways to detect the end point when the reaction is complete.

1 Color change indicators

The method may utilize an indicator which changes color when the reaction is complete. For example, in the Chemical Oxygen Demand Test the indicator, ferroin, gives a blue-green color to the mixture until the oxidation-reduction reaction is complete. Then the mixture is reddish-brown.

Several of these color-change titrations make use of the iodometric process whereby the constituent of interest quantitatively releases free iodine. Starch is added to give a blue color until enough reducing agent (sodium thiosulfate or phenylarsine oxide) is added to react with all the iodine. At this end point, the mixture becomes colorless.

2 Electrical property indicators

Another way to detect end points is a change in an electrical property of the solution when the reaction is complete. In the chlorine titration a cell containing potassium chloride will produce a small direct current as long as free chlorine is present. As a reducing agent (phenylarsine oxide) is added to reduce the chlorine, the microammeter which measures the existing direct current registers a lower reading on a scale. By observing the scale, the end point of total reduction of chlorine can be determined because the direct current ceases.

3 Specified end points

For acidity and alkalinity titrations, the end points are specified pH values for the final mixture. The pH values are those existing when common acidity or alkalinity components have been neutralized. Thus acidity is determined by titrating the sample with a standard alkali to pH 8.2 when carbonic acid would be neutralized to $(HCO_3)^-$. Alkalinity (except for highly acidic samples) is determined by titrating the sample with a standard acid to pH 4.5 when the carbonate present has been converted to carbonic acid. pH meters are used to detect the specified end points.

B Gravimetric Procedures

Gravimetric methods involve direct weighing of the constituent in a container. An empty container is weighed, the constituent is separated from the sample mixture and isolated in the container, then the container with the constituent is weighed. The difference in the weights of the container before and after containing the constituent represents the weight of the constituent.

The type of container depends on the method used to separate the constituent from the sample mixture. In the solids determinations, the container is an evaporating dish (total or dissolved) or a glass fiber filter disc in a crucible (suspended). For oil and grease, the container is a flask containing a residue after evaporation of a solvent.

C Combustion

Combustion means to add oxygen. In the Total Organic Carbon Analysis, combustion is used within an instrument to convert carbonaceous material to carbon dioxide. An infrared analyzer measures the carbon dioxide.

VI PHOTOMETRIC METHODS

These methods involve the measurement of light that is absorbed or transmitted quantitatively either by the constituent of interest or else by a substance containing the constituent of interest which has resulted from some treatment of the sample. The quantitative aspect of these photometric methods is based on applying the Lambert-Beer Law which established that the amount of light absorbed is quantitatively related to the concentration of the absorbing medium at a given wavelength and a given thickness of the medium through which the light passes.

Each method requires preparing a set of standard solutions containing known amounts of the constituent of interest. Photometric values are obtained for the standards. These are used to draw a calibration (standard) curve by plotting photometric values against the concentrations. Then, by locating the photometric value for the sample on this standard curve, the unknown concentration in the sample can be determined.

A Atomic Absorption

Atomic Absorption (AA) instruments utilize absorption of light of a characteristic wavelength. This form of analysis involves aspirating solutions of metal ions (cations) or molecules containing metals into a flame where they are reduced to individual atoms in a ground electrical state. In this condition, the atoms can absorb radiation of a wavelength characteristic for each element. A lamp containing the element of interest as the cathode is used as a source to emit the characteristic line spectrum for the element to be determined.

The amount of energy absorbed is directly related to the concentration of the element of interest. Thus the Lambert-Beer Law applies. Standards can be prepared and tested and the resulting absorbance values can be used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Once the instrument is adjusted to give optimum readings for the element of interest, the testing of each solution can be done in a matter of seconds. Many laboratories wire recorders into their instruments to rapidly transcribe the data, thus conserving time spent on this aspect of the analysis. Atomic absorption techniques are generally used for metals and semi-metals in solution or else solubilized through some form of sample processing. For mercury, the principle is utilized but the absorption of light occurs in a flameless situation with the mercury in the vapor state and contained in a closed glass cell.

B Flame Emission

Flame emission photometry involves measuring the amount of light given off by atoms drawn into a flame. At certain temperatures, the flame raises the electrons in atoms to a higher energy level. When the electrons fall back to a lower energy level, the atoms lose (emit) radiant energy which can be detected and measured.

Again standards must be prepared and tested to prepare a calibration (standard) curve. Then the transmission value of the sample can be located on the curve to determine its concentration.
Many atomic absorption instruments can be used for flame emission photometry. Sodium and potassium are very effectively determined by the emission technique.

However, for many elements, absorption analysis is more sensitive because there are a great number of unexcited atoms in the flame which are available to absorb the radiant energy.

C Colorimetry

Colorimetric analyses involve treating standards which contain known concentrations of the constituent of interest and also the sample with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color.

The Lambert-Beer Law which relates the absorption of light to the thickness and concentration of the absorbing medium applies. Accordingly, a spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by the same thickness of each solution. The results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Many of the metals and several other parameters (phosphorus, ammonia, nitrate, nitrite, etc.) are determined in this manner.

VII GAS-LIQUID CHROMATOGRAPHY

Chromatography techniques involve a separation of the components in a mixture by using a difference in the physical properties of the components. Gas-Liquid Chromatography (GLC) involves separation based on a difference in the properties of volatility and solubility. The method is used to determine algicides, chlorinated organic compounds and pesticides.

The sample is introduced into an injector block which is at a high temperature (e.g. 210°C), causing the liquid sample to volatilize. An inert carrier gas transports the sample components through a liquid held in place as a thin film on an inert solid support material in a column. Sample components pass through the column at a speed partly governed by the relative solubility of each in the stationary liquid. Thus the least soluble components are the first to reach the detector. The type of detector used depends on the class of compounds involved. All detectors function to sense and measure the quantity of each sample component as it comes off the column. The detector signals a recorder system which registers a response.

As with other instrumental methods, standards with known concentrations of the substance of interest are measured on the instrument. A calibration (standard) curve can be developed and the concentration in a sample can be determined from this graph.

Gas-liquid chromatography methods are very sensitive (nanogram, picogram quantities) so only small amounts of samples are required. On the other hand, this extreme sensitivity often necessitates extensive clean-up of samples prior to GLC analysis.

VIII AUTOMATED METHODS

The increasing number of samples and measurements to be made in water quality laboratories has stimulated efforts to automate these analyses. Using smaller amounts of sample (semi-micro techniques), combining reagents for fewer measurements per analysis, and using automatic dispensers are all means of saving analytical time.

However, the term "automated laboratory procedures" usually means automatic introduction of the sample into the instrument, automatic treatment of the sample to test for a component of interest, automatic recording of data and, increasingly, automatic calculating and print-out of data. Maximum automation systems involve continuous sampling direct from the source (e.g. an in-place probe) with telemetering of results to a central computer.

Automated methods, especially those based on colorimetric methodology. are recognized for several water quality parameters including alkalinity, ammonia, nitrate, nitrite, phosphorus, and hardness.

IX SOURCES OF PROCEDURES

Details of the procedure for an individual measurement can be found in reference books. There are three particularly-recognized books of procedures for water quality measurements.

A Standard Methods(1)

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish "Standard Methods for the Examination of Water and Wastewater." As indicated by the list of publishers, this book contains methods developed for use by those interested in water or wastewater treatment.

B ASTM Standards(2)

The American Society for Testing and Materials publishes an "Annual Book of ASTM Standards" containing specifications and methods for testing materials. The "book" currently consists of 47 parts. The part applicable to water was formerly Part 23. It is now Part 31, Water.

The methods are chosen by approval of the membership of ASTM and are intended to aid industry, government agencies and the general public. Methods are applicable to industrial waste waters as well as to other types of water samples.

C EPA Methods Manual(3)

The United States Environmental Protection Agency publishes a manual of "Methods for Chemical Analysis of Water and Wastes."

EPA developed this manual to provide methodology for monitoring the quality of our Nation's waters and to determine the impact of waste discharges. The test procedures were carefully selected to meet these needs, using Standard Methods and ASTM as basic references. In many cases, the EPA manual contains completely described procedures because they modified methods from the basic references. Otherwise, the manual cites page numbers in the two references where the analytical procedures can be found.

X ACCURACY AND PRECISION

A Of the Method

One of the criteria for choosing methods to be used for water quality analysis is that the method should measure the desired property or constituent with precision, accuracy, and specificity sufficient to meet data needs. Standard references, then, include a statement of the precision and accuracy for the method which is obtained when (usually) several analysts in different laboratories used the particular method.

B Of the Analyst

Each analyst should check his own precision and accuracy as a test of his skill in performing a test. According to the U. S. EPA Handbook for Analytical Quality Control(4), he can do this in the following manner.

To check precision, the analyst should analyze samples with four different concentrations of the constituent of interest, seven times each. The study should cover at least two hours of normal laboratory operations to allow changes in conditions to affect the results. Then he should calculate the standard deviation of each of the sets of seven results and compare his values for the lowest and highest concentrations tested with the standard deviation value published for that method in the reference book. **(It may be stated as % relative standard deviation. If so, calculate results in this form.) An individual should have better values than those averaged from the work of several analysts.**

To check accuracy, he can use two of the samples used to check precision by adding a known amount (spike) of the particular constituent in quantities to double the lowest concentration used, and to bring an intermediate concentration to approximately 75% of the upper limit of application of the method. He then analyzes each of the spiked samples seven times, then calculates the average of each set of seven results. To calculate accuracy in terms of % recovery, he will also need to calculate the average of the results he got when he analyzed the unspiked samples (background). Then:

$$\% \text{ Recovery} = \left[\frac{\text{observed - background}}{\text{spike}}\right] \times 100$$

The actual calculation involves volume-concentration calculations for each term.

If the published accuracy is stated as % bias, subtract 100% from % recovery to compare results. Again, the individual result should be better than the published figure derived from the results of several analysts.

C Of Daily Performance

Even after an analyst has demonstrated his personal skill in performing the analysis, a daily check on precision and accuracy should be done. About one in every ten samples should be a duplicate to check precision and about one in every ten samples should be spiked to check accuracy.

It is also beneficial to participate in interlaboratory quality control programs. The U.S. EPA provides reference samples at no charge to laboratories. These samples serve as independent checks on reagents, instruments or techniques; for training analysts or for comparative analyses within the laboratory. There is no certification or other formal evaluative function resulting from their use.

XI SELECTION OF ANALYTICAL PROCEDURES

Standard sources$^{(1, 2, 3)}$ will, for most parameters, contain more than one analytical procedure. Selection of the procedure to be used in a specific instance involves consideration of the use to be made of the data. In some cases, one must use specified procedures. In others, one may be able to choose among several methods.

A NPDES Permits and State Certifications

A specified analytical procedure must be used when a waste constituent is measured:

1 For an application for a National Pollutant Discharge Elimination System (NPDES) permit under Section 402 of the Federal Water Pollution Control Act (FWPCA), as amended.

2 For reports required to be submitted by dischargers under NPDES.

3 For certifications issued by States pursuant to Section 401 of the FWPCA, as amended.

Analytical procedures to be used in these situations must conform to those specified in Title 40, Chapter 1, Part 136, of the Code of Federal Regulations (CFR). The listings in the CFR usually cite two different procedures for a particular measurement.

The CFR also provides a system of applying to EPA for permission to use methods not cited in the CFR. Approval of alternative methods for nationwide use will be published in the Federal Register.

B Ambient Water Quality Monitoring

For Ambient Water Quality Monitoring, analytical procedures have not been specified by regulations. However, the selection of procedures to be used should receive attention. Use of those listed in the CFR is strongly recommended. If any of the data obtained is going to be used in connection with NPDES permits, or may be used as evidence in a legal proceeding, use of procedures listed in the CFR is again strongly recommended.

C Drinking Water Monitoring

In December, 1975, National Interim Primary Drinking Water Regulations to be effective June 24, 1977 were published in the Federal Register in Title 40, Chapter 1, Subchapter D, Part 141. The publication includes specification of analytical procedures to be used when determining compliance with the maximum contaminant levels of required parameters.

Because of the low concentrations involved in the regulations, there is often just one analytical method cited for each parameter.

Individuals or organizations may apply to EPA for permission to use methods not cited in the above. Approval of alternative methods for nationwide use will be published in the Federal Register.

XII FIELD KITS

Field kits have been devised to perform analyses outside of the laboratory. The kit may contain equipment and reagents for only one test or for a variety of measurements. It may be purchased or put together by an agency to serve its particular needs.

Since such kits are devised for performing tests with minimum time and maximum simplicity, the types of labware and reagents employed usually differ significantly from the equipment and supplies used to perform the same measurement in a laboratory.

A Shortcomings

Field conditions do not accommodate the equipment and services required for pretreatments like distillation and digestion. Nor is it practical to carry and use calibrated glassware like burets and volumetric pipets. Other problems are preparation, transport and storage of high quality reagents, of extra supplies required to test for and remove sample interferences before making the measurement, and of instruments which are very sensitive in detecting particular constituents. One just cannot carry and set up laboratory facilities in the field which are equivalent to stationary analytical facilities.

B Uses

Even though the results of field tests are usually not as accurate and precise as those performed in the laboratory, such tests do have a place in water quality programs.

In situations where only an estimate of the concentrations of various constituents is required, field tests serve well. They are invaluable sources of information for planning a full-scale sampling/testing program when decisions must be made regarding location of sampling sites, schedule of sample collection, dilution of samples required for analysis, and treatment of samples required to remove interferences to analyses.

C NPDES Permits and State Certification

Kit methods are not approved for obtaining data required for NPDES permits or State construction certifications. If one judges that such a method is justifiable for these purposes, he must apply to EPA for permission to use it.

D Drinking Water Monitoring

The DPD test kit for residual chlorine is approved in the December, 1975 Federal Register for monitoring drinking water.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 14th Edition. 1976, APHA-AWWA-WPCF, 1015 18th Street, N.W., Washington, D.C. 20036

2 1975 Annual Book of ASTM Standards, Part 31, Water. ASTM, 1916 Race Street, Philadelphia, PA 19103.

3 Methods for Chemical Analysis of Water and Wastes. 1979, U. S. EPA, EMSL, Cincinnati, OH 45268.

4 Handbook for Analytical Quality Control in Water and Wastewater Laboratories, 1972. U. S. EPA, EMSL, Cincinnati, Ohio 45268.

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Descriptors: Analysis, Chemical Analysis, Methodology, Wastewater, Water Analysis

STATISTICS FOR CHEMISTS

I INTRODUCTION

A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.

B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

II FREQUENCY

A Definitions

1 Frequency - indicates how many times a particular score occurs in a collection of data

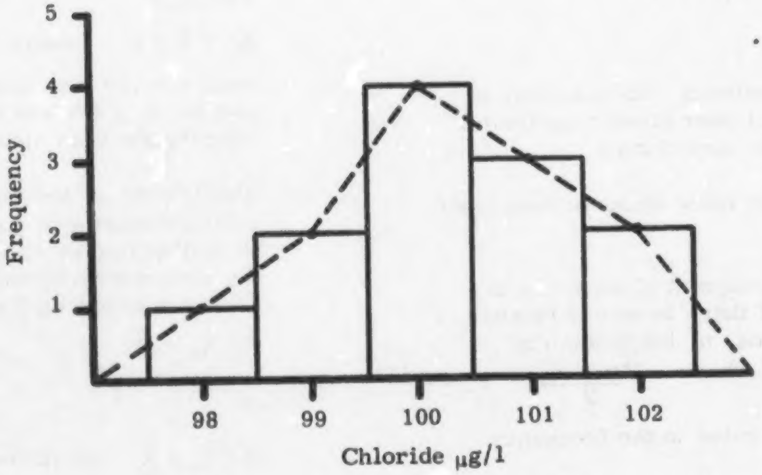
2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies

3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)

4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting midpoints of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results (µg/l)

100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride (µg/l)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

1 Central tendency - the tendency of values to cluster about a particular value in the distribution

2 Mode - that value which occurs most frequently

3 Median - midpoint of an array of scores. If there is an odd number of observations, n, the median is $X_{\frac{n+1}{2}}$ where $X_{\frac{n+1}{2}}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2}$, the average of the middle two scores.

4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\bar{X}$. The formula for calculating the sample mean is

$$\bar{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$\bar{X} = \frac{\Sigma X_i}{n}$ where there are n number of values.

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:

$\bar{X}_c = \bar{X} \pm C$ where the X_i's are the values in the distribution with mean $\bar{X}$, and the $X_i \pm C$'s are the values in the distribution with mean $\bar{X}_c$.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

(1) $\bar{X}_c = C\bar{X}$

or

(2) $\bar{X}_c = \frac{\bar{X}}{C}$ where the X_i's are the values in the distribution with mean $\bar{X}$,

and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\overline{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

2 $$\text{Median} = \frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2} = \frac{X_6 + X_7}{2}$$

$$= \frac{100 + 100}{2} = 100$$

3 $$\text{Mean} = \frac{\Sigma X_i}{n}$$

$$= \frac{98 + 2\,(99) + 4\,(100) + 3\,(101) + 2\,(102)}{12}$$

$$= 100.25$$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2

Frequency Table

Chloride (µg/l)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\overline{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, X, of the distribution in Table 2, we obtain the mean, $\overline{X}_c$, of the distribution in Table 1.

Thus $\overline{X}_c = \overline{X} + 100$

$$\overline{X}_c = \frac{\Sigma X}{n} + 100$$

$$\overline{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$$\overline{X}_c = .25 + 100 = 100.25$$

IV MEASURES OF DISPERSION

A Definitions

1 Dispersion - spread or variability of observations in a distribution

2 Range - the difference between the highest value and the lowest value

$$R = \max - \min$$

3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\Sigma |X_i - \overline{X}|}{n}$$

4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\Sigma(X_i - \overline{X})^2}{n - 1}$$

6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$$ where there are n number of values.

7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\overline{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V), or % relative standard deviation:

$$V = \frac{s}{\overline{X}} \times 100$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

(1) $s_c^2 = s^2$

(2) $s_c = s$

where the X_i's are the values in the distribution with variance s^2 and standard deviation s, and the $X_i \pm C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

(1) $s_c^2 = C^2 s^2$

(2) $s_c^2 = \frac{s^2}{C^2}$

where the X_i's are the values in the distribution with variance s^2, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2.

3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

(1) $s_c = Cs$
or
(2) $s_c = \frac{s}{C}$

where the X_i's are the values in the distribution with standard deviation s, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c.

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

1 Range = 102 - 98 = 4

2 Average deviation - $d = \frac{\Sigma|X_i - \bar{X}|}{n}$

n	X_i	$\|X_i - \bar{X}\|$	$n\|X_i - \bar{X}\|$
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
$\bar{X} = 100.25$			11.50

$$d = \frac{\Sigma|X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

4 Variance - $s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n-1}$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	-.25	.06	.24
3	101	+.75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n - 1} = \frac{16.22}{11} = 1.47$$

5 Standard deviation - $s = \sqrt{\dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n - 1}}$

n	X_i	nX_i	X^2_i	nX^2_i
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\frac{120617 - \frac{1203^2}{12}}{11}} = \sqrt{\frac{120617 - 120601}{11}}$$

$$s = \sqrt{\frac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

n	X_i-C	$n(X_i$-C)	$(X_i$-C$)^2$	$n(X_i$-C$)^2$
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}$$

$$s^2 = \frac{17 - \frac{(3)^2}{12}}{11} = \frac{16.25}{11} = 1.48$$

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \frac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \frac{s}{\sqrt{n}} = \frac{1.22}{\sqrt{12}} = \frac{1.22}{3.46} = 0.\text{[illegible]}$$

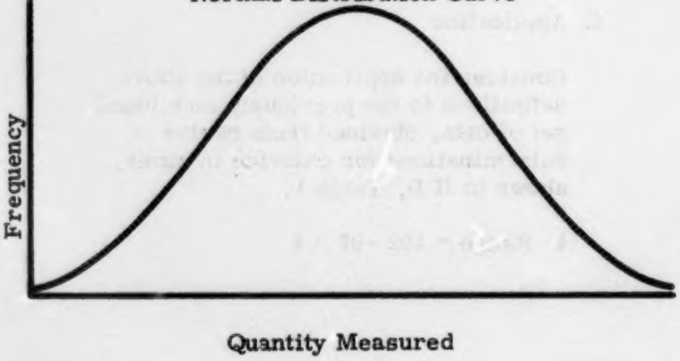
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \frac{s}{\bar{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\bar{X} = 100.25$,

$$V = \frac{s}{\bar{X}} = \frac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

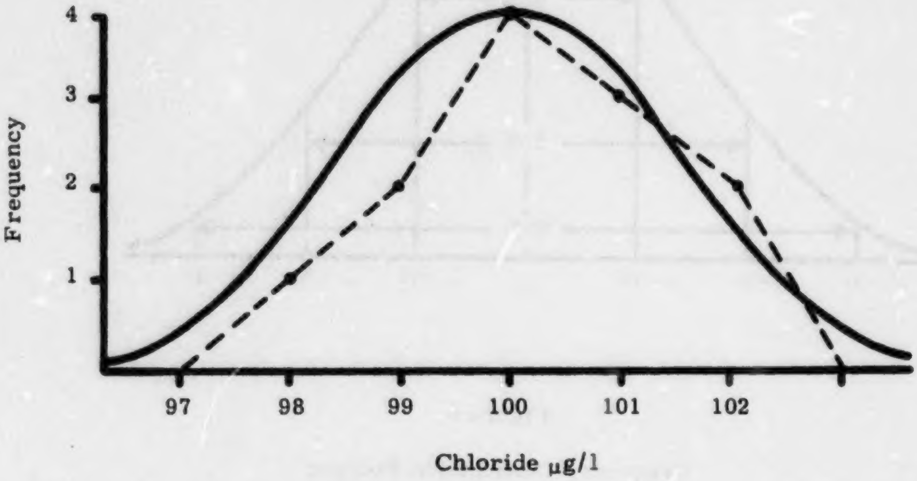
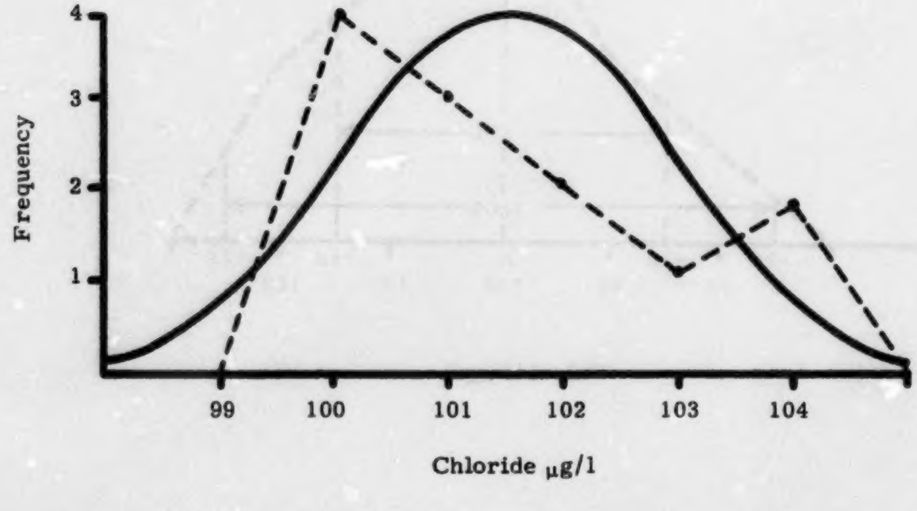


Figure 4

Comparison of Normal Curve and Frequency Polygon



B If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.

Figure 5 shows the normal distribution in terms of the population mean μ, and the standard deviation of the population σ, and gives the percent of area under the curve between certain points.

Figure 5

Normal Distribution Curve

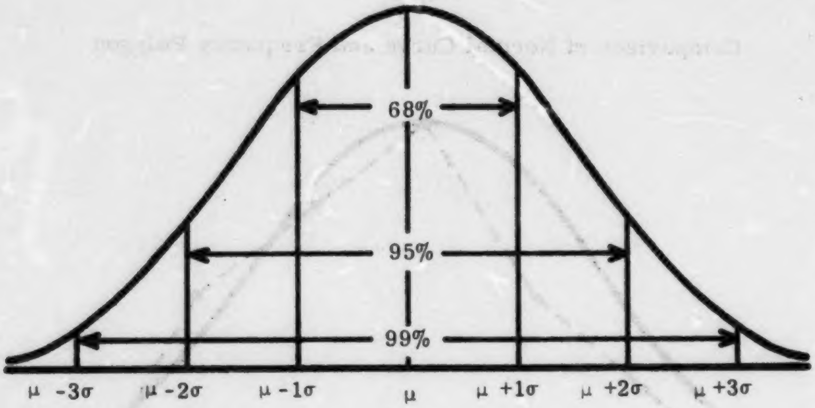
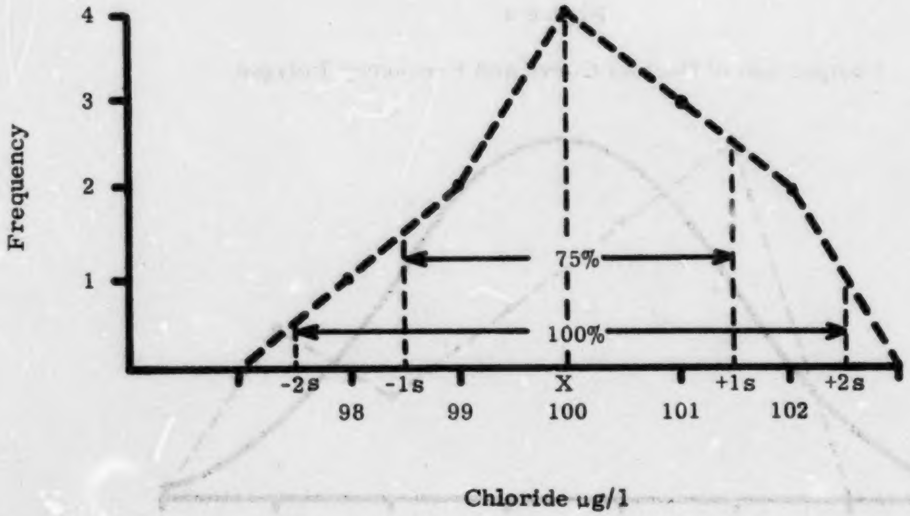


Figure 6

Frequency Distribution Polygon



We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\overline{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\overline{X}$ = 100, See III C 3). The value of the sample standard deviation (s = 1.21, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\overline{X} \pm 1s$, $\overline{X} - 1s = 98.79$ and $\overline{X} + 1\ s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2\sigma$'s from the population mean. In fact, 100% of the observations were within $\pm$ 2 s's from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

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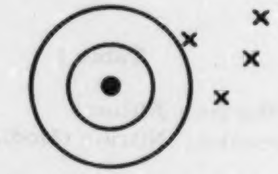
Descriptors: Graphic Methods, Quality Control, Statistical Methods, Statistics

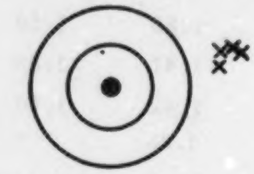
ACCURACY-PRECISION-ERROR

I INTRODUCTION

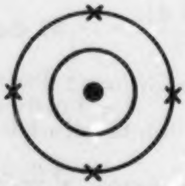
An analytical method is subject to errors. These errors may affect the accuracy of the method because they introduce bias into the results. There are other types of errors which affect the precision of the method because they produce random fluctuations in the data. The most desirable situation for the analyst is shown in the diagram in Figure 1 where the results are both precise and accurate.



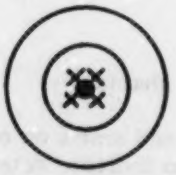
IMPRECISE AND INACCURATE



PRECISE BUT INACCURATE



ACCURATE BUT IMPRECISE



PRECISE AND ACCURA

Figure 1. PRECISION AND ACCURACY

A Accuracy

For results to be accurate, the analysis used must give values close to the true value.(See Figure 1)

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. It is a measure of the degree to which results "check." (See Figure 1)

C Note

It is possible to have precision without accuracy. (See Figure 1)

II DETERMINATE ERROR AND ACCURACY

A determinate error is one which contributes a constant error or bias to results, causing them to be inaccurate. This constant error makes it possible for results to agree with each other (be precise) and still be inaccurate.(See Figure 1)

Determinate errors have "assignable" causes which can usually be identified and either eliminated or controlled. (The terms "determinate" error, "assignable" error, and "systematic" error are synonymous).

A Sources of Determinate Error

1 Method error

Method errors are those that are inherent in the procedure. These are very serious and the hardest to detect and correct. The most common method error is the presence of interferences in the sample. Other examples would be precipitation of substances other than the desired material, partial solubility of precipitates, and entrainment as in a solvent extraction procedure.

2 Personal errors

Personal errors are attributable to individual mistakes which are consistently made by an analyst. These errors are the result of consistent carelessness, lack of knowledge or personal bias. Examples are errors in calculations, use of contaminated reagents, non-representative sampling, or poor calibration of standards and instruments.

3 Instrumental errors

Instrumental errors are those which are caused by an analytical instrument or by the effects of the environment acting on the instrument. Moisture in a G. C. column, improper wavelength markings on a spectrophotometer or incorrect scoring on a buret would be examples.

B Effects of Determinate Error

1 Additive

An additive determinate error is one which has a constant value regardless of the amount of analytically sought constituent present in the sample. (See Figure 2)

2 Proportional

A proportional determinate error changes value according to the amount of analytically sought constituent in the sample. (See Figure 3)

III DETECTION OF DETERMINATE ERROR

A Spiked Samples

1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

a Mean error - the difference between the mean of the data and the true result.

b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.50 mg/1. He then adds 1 mg/1 of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample, and calculation of both mean and relative errors.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

Xi	
1.35	1.56
1.47	1.59
1.49	1.60
1.55	

1 Calculation of mean error

$$\overline{X} = \frac{10.61}{7} = 1.51 \text{ mg/l}$$

$$\text{Mean Error} = 1.51 - 1.50 = +0.01 \text{ mg/1}$$

2 Calculation of relative error

$$\% \text{ Relative Error} = \frac{+0.01 \times 100}{1.50} = +0.7\,\%$$

---

2 Control charts

Trends and shifts on control charts may also indicate determinant error. Using spiked samples, the standard deviation is calculated and control limits (usually ± 3 standard deviations) for the analysis are set (see Figure 4. For further discussion of control limits, see reference 3, p. 62.

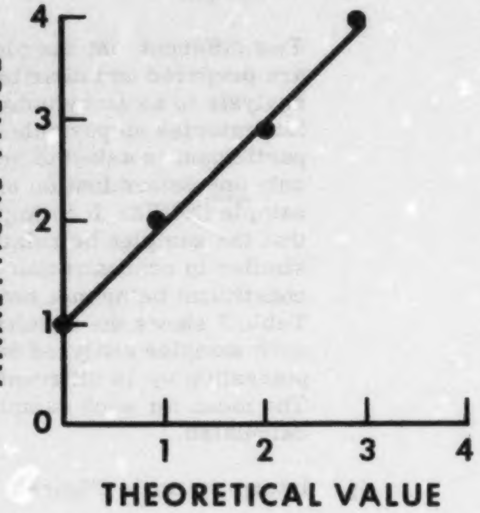


Figure 2. ADDITIVE ERROR

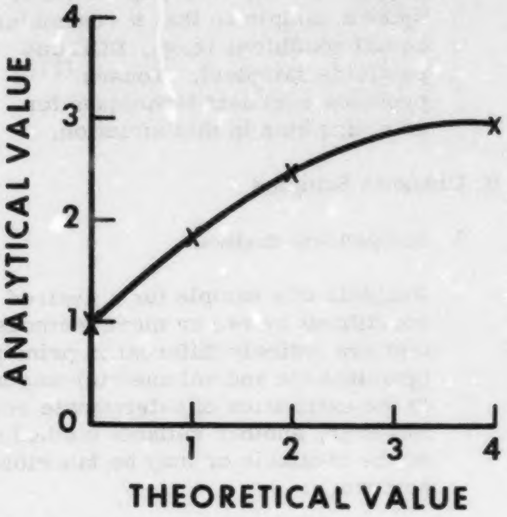


Figure 3. PROPORTIONAL ERROR

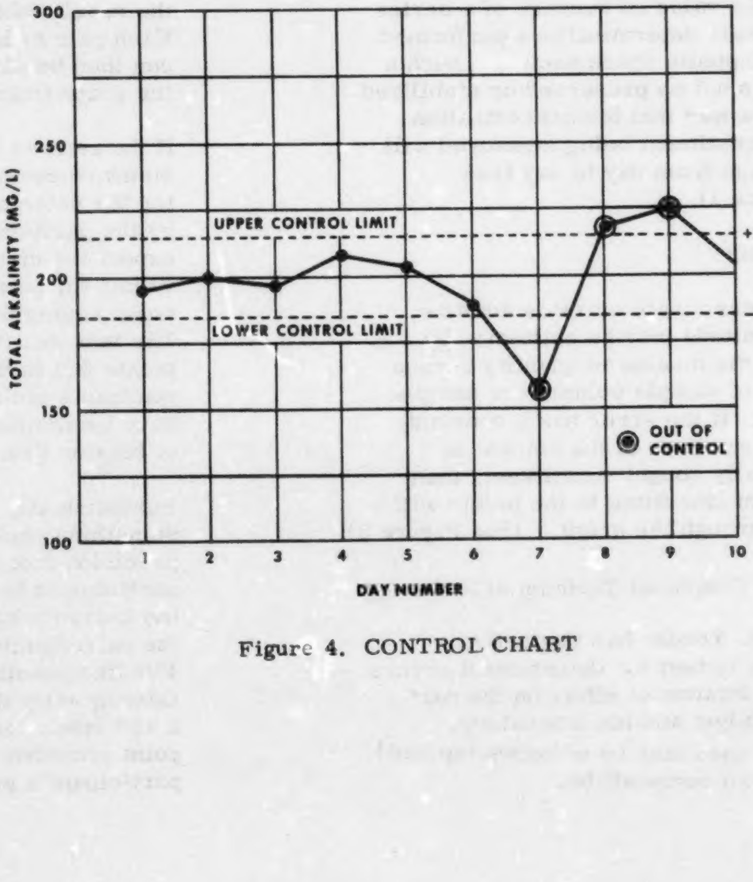


Figure 4. CONTROL CHART

3 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden (11) provides excellent techniques for detecting bias in this situation.

B Unknown Samples

1 Independent method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error. However, another reliable method may not be available or may be laborious to perform.

2 Control charts

It is possible to plot a control chart (Figure 4) even when it is not possible to spike a sample. One can use as a reference value an average of a series of replicate determinations performed on a composite check sample. Such a sample must be preserved or stabilized in such a way that the concentration of the constituent being measured will not change from day to day (see reference 1).

3 Aliquoting

If the determinate error is additive, the magnitude may be estimated by plotting the measured quantity versus a range of sample volumes or sample weights. If the error has a constant value regardless of the amount of analytically sought constituent, then a straight line fitted to the points will not go through the origin. (See Figure 5)

C Youden's Graphical Technique (10, 11, 12)

Dr. W. J. Youden has devised an approach to test for determinant errors with a minimum of effort on the part of the analyst and his laboratory. Samples used may be of known (spiked) or unknown composition.

1 Technique

Two different test samples (X and Y) are prepared and distributed for analysis to as many individuals or laboratories as possible. Each participant is asked to perform only one determination on each sample (NOTE: It is important that the samples be relatively similar in concentration of the constituent being measured). Table 2 shows the results on two such samples analyzed for percent potassium by 14 different laboratories. The mean for each sample has been calculated.

2 Interpretation - Figure 6

The vertical line drawn on the graph represents the mean $(\overline{X})$ of all the results obtained on Sample X; the horizontal line was drawn through the mean $(\overline{Y})$ of all the results obtained on sample Y. Each pair of laboratory results can then be plotted as a point on the graph (marked X. 1 etc.)

If the ratio of the bias (error) to standard deviation is close to zero for the determinations submitted by the participants, then one would expect the distribution of the paired values (or points) to be close to equal among the four quadrants. The fact that the majority of the points fall in the (+, +) and (-, -) quadrants indicates that the results have been influenced by some source of bias or determinate error.

Furthermore, one can even learn something about a participant's precision from the graph. If all participants had perfect precision (no indeterminate error), then all the paired points would fall on a 45° line passing through the origin. Consequently the distance from such a 45° line of each participant's point provides an indication of that participant's precision.

3. **Examples of Youden two-sample charts are in the Appendix (p4-13).**

4. **A quantitative treatment of this subject can be found in references 11 and 12.**

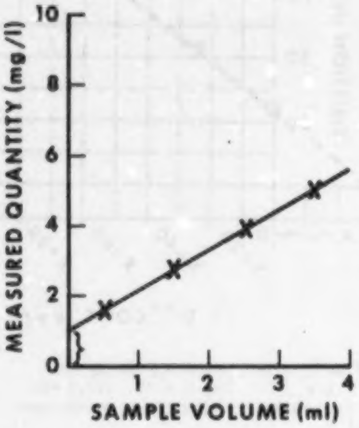


Figure 5

Table 2

Laboratory	Sample X	Sample Y
1	9.74%	8.50%
2	9.92	8.28
3	9.98	8.84
4	9.99	8.24
5	10.00	8.73
6	10.11	8.54
7	10.12	8.64
8	10.14	8.82
9	10.19	9.04
10	10.23	8.93
11	10.25	8.97
12	10.29	8.80
13	10.55	9.21
14	10.62	8.95

$\overline{X} = 10.15$ $\overline{Y} = 8.75$

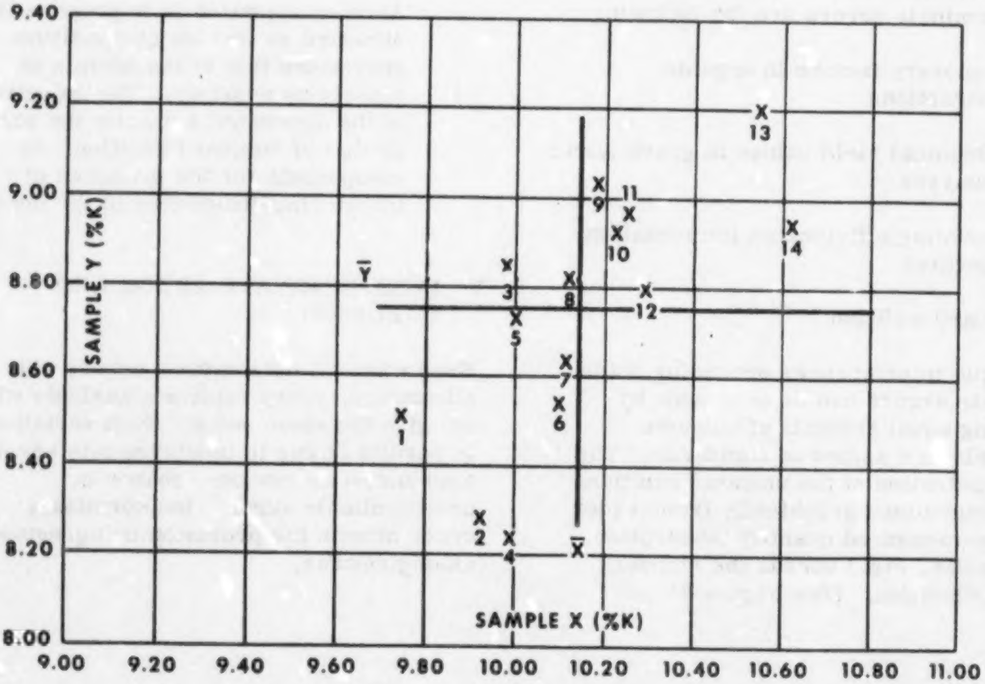


Figure 6. YOUDEN'S GRAPHICAL TECHNIQUE

IV ELIMINATION OF DETERMINATE ERROR

Various approaches can be used to eliminate the source of determinate error. The approach used depends upon whether the source is personal, method, or instrumental.

A Personal

Great care must be used to avoid producing an unconfident attitude in a technician. It is undesirable for the analyst to feel he is being "policed."

B Method and Instrumental

1 Blanks

Blanks can be used to correct for interferences from reagents, sample color, etc.

2 Correction factors

Examples of correction factors used in environmental analyses to eliminate determinate errors are the following:

a Recovery factors in organic extractions

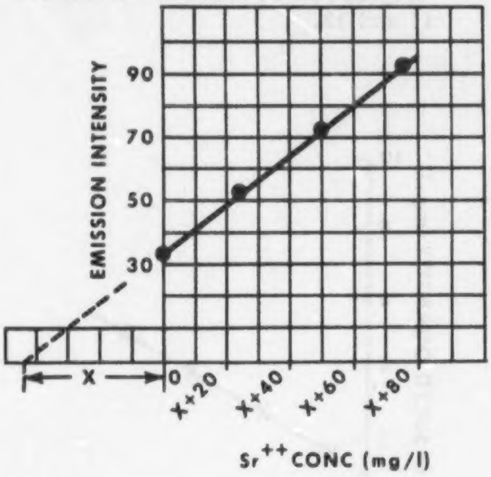
b Chemical yield values in gravimetric analyses

c Counting efficiencies for radiation counters

3 Standard addition

Sample interferences producing determinate errors can be overcome by adding equal amounts of unknown sample to a series of standards. The concentration of the unknown can then be determined graphically from a plot of the measured quantity (absorption, emission, etc.) versus the standard concentration. (See Figure 7)

Figure 7. METHOD OF STANDARD ADDITION GRAPHICAL METHOD OF COMPUTING Sr CONCENTRATION



NOTE: The X value appearing along the abscissa (i.e., X + 20, X + 40, etc.) refers to the unknown added to the strontium standard

4 Standard compensation

Another approach is to prepare the standard so that its composition resembles that of the sample as closely as possible. The objective of the approach is exactly the same as that of standard addition - to compensate for the presence of interfering substances in the unknown.

V INDETERMINATE ERROR AND PRECISION

Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Such variation in results is due to indeterminate error, also known as random, chance or uncontrollable error. Indeterminate error affects the precision or agreement among results.

A Sources of Indeterminate Error

Indeterminate errors are due to unassignable or "chance causes." Examples are inadvertent contamination of sample or glassware, variation in reagent additions, or variations in instrument response (see Figure 8).

B Effects of Indeterminate Error

Since the causes of these errors are random, the effects are also random. Fortunately, these random variations conform to the "Laws of Chance" so statistical measures of precision can be used to quantitate indeterminate errors.

VI DETECTION OF INDETERMINATE ERROR

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

An estimation of indeterminate error can be obtained through a calculation of the standard deviation. The following formulas should be applied to random data which follows a normal distribution. Normality can be checked by ranking and plotting the data on normal probability paper; it should fall on a straight line (see Figure 9). Any values which do not fall close to the straight line (those values encircled) should be rejected in the calculation of standard deviation. Other statistical tests (8) can be used to objectively evaluate the rejection of outliers. The value obtained for standard deviation for a particular method may vary with the analyst, the concentration range of the constituent, and the composition of the sample analyzed. The confidence of the estimate is increased as the number of results (n) used to compute the standard deviation is increased.

1 Replicate results on the same sample:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n-1}} \qquad (1)$$

X_i = value of single result

$\overline{X}$ = average (mean) of results on same sample

n = number of results

Example: Table 3 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a colaborative study. Calculate the standard deviation of these results.

$\overline{X}$ = 192 mg/l

n = 36.0

$\Sigma(X_i - \overline{X})^2$ = 58, 200

$$s = \sqrt{\frac{58,200}{35}}$$

s = 41 mg/l

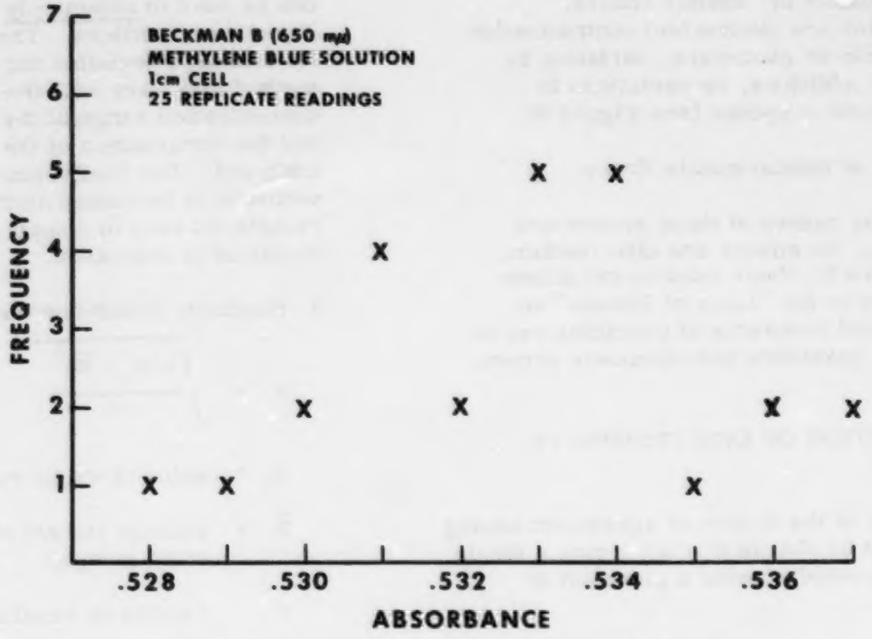


Figure 8. VARIATIONS IN SPECTROPHOTOMETER READINGS

FIGURE 9 NORMAL PROBABILITY CURVE

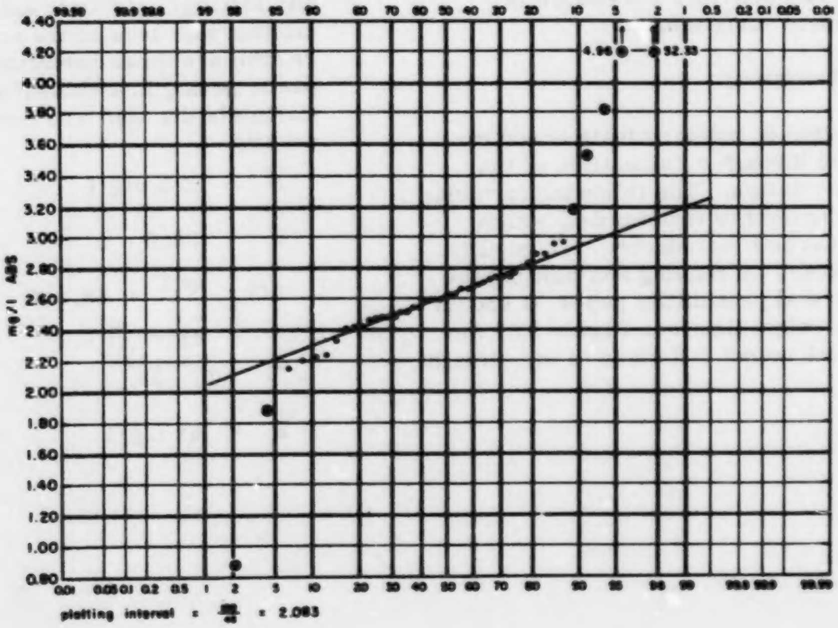


Table 3

SAMPLE: 150 mg/l glucose + 150 mg /l glutamic acid (1% dilution)
DETERMINATION: 5-day Biochemical Oxygen Demand

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
100 mg/l	-92 mg/l	8464 (mg/l)^2	198 mg/l	6 mg/l	36 (mg/l)^2
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	- 3	9	238	46	2116
190	- 2	4	247	55	3025
196	4	16	250	58	3344
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R.A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Table 4*

SAMPLE: Aqueous
CONCENTRATION RANGE: 30 - 70 mg/l
DETERMINATION: Phosphate (Lucerna, Cende, and Prat Method)

Sample	Mg/l P	d	d^2	Sample	Mg/l P	d	d^2
A	51 53	2	4	L	54 56	2	4
B	39 39	0	0	M	38 37	1	1
C	53 54	1	1	N	52 52	0	0
D	47 47	0	0	O	58 58	0	0
E	50 51	1	1	P	54 54	0	0
F	48 47	1	1	Q	54 54	0	0
G	50 50	0	0	R	48 48	0	0
H	47 47	0	0	S	52 51	1	1
I	42 42	0	0	T	53 53	0	0
J	50 51	1	1	U	46 47	1	1
K	59 60	1	1	V	42 42	0	0

*Data obtained from Frank Schickner, Proctor and Gamble Company.

2 Duplicate results on different samples

In a laboratory where duplicates are routinely run, it would be simple to use the following formula for evaluation of standard deviation.

$$s = \sqrt{\frac{\Sigma(d^2)}{2k}} \quad \text{(See reference 5, page 654)} \qquad (2)$$

d = difference between duplicates

k = number of samples

Example: Table 4 contains a set of phosphate results obtained on aqueous samples in the range 100-200 mg/l. Calculate the standard deviation of these results.

$$\Sigma(d^2) = 16$$

$$k = 22$$

$$s = \sqrt{\frac{16}{44}}$$

$$s = .61 \text{ mg/l}$$

3 Duplicate and triplicate results on different samples

$$s = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - k}} \quad \text{(See reference 7, page 73)} \qquad (3)$$

$\bar{x}$ = average of results on the same sample

n = total number of results

k = number of different samples

Example: Table 5 contains a set of % nitrogen results obtained on unknown organic compounds.

Calculate the standard deviation.

$$\Sigma(X_i - \bar{X})^2 = .2401$$

$$n - k = 22$$

$$s = \sqrt{\frac{.2401}{22}}$$

$$s = .104\%$$

4 Use of range to estimate standard deviation

For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (See Table 6).

$$\frac{R}{d_N} = s \qquad (4)$$

Example: Table 7 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formula (1)

$$\bar{X} = 0.72 \text{ mg/l}$$

$$n = 5$$

$$\Sigma(X_i - \bar{X})^2 = .0134$$

$$s = \sqrt{\frac{.0134}{4}}$$

$$s = .058 \text{ mg/l}$$

b Use of formula (4)

$$R = .14 \text{ mg/l}$$

$$d_N = 2.33$$

$$s = \frac{.14 \text{ mg/l}}{2.33}$$

$$s = .060 \text{ mg/l}$$

C Coefficient of Variation

An estimation of indeterminate error can also be made by calculating the coefficient of variation (V), also known as % relative standard deviation:

$$V = \frac{s}{\bar{X}} \times 100$$

By comparing the standard deviation (dispersion) to the average or mean value (central tendency) in a set of data and expressing this relative standard deviation as a percentage, the analyst has a meaningful interpretation of the degree of dispersion present. Indeter-

Table 5*

SAMPLE: Unknown Organic Compounds
DETERMINATION: % Nitrogen (Kjeldahl)

CONCENTRATION RANGE: 10% - 20%

Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
A	16.48	16.47	.01	.0001
	16.46		.01	.0001
B	16.50	16.50	0	0
	16.49		.01	.0001
C	16.72	16.65	.07	.0049
	16.57		.08	.0064
D	17.52	17.58	.06	.0036
	17.60		.02	.0004
	17.63		.05	.0025
E	16.31	16.31	0	0
	16.30		.01	.0001
F	16.40	16.35	.05	.0025
	16.31		.04	.0016
	16.35		0	0
G	17.56	17.55	.01	.0001
	17.54		.01	.0001
H	14.96	14.81	.15	.0225
	14.66		.15	.0225
I	19.15	19.02	.13	.0169
	18.89		.13	.0169
J	13.93	13.75	.18	.0324
	13.56		.19	.0361
K	10.34	10.27	.07	.0049
	10.19		.08	.0064
L	17.16	17.15	.01	.0001
	17.13		.02	.0004
M	15.01	15.03	.02	.0004
	15.05		.02	.0004
N	12.44	12.62	.18	.0324
	12.70		.08	.0064
	12.73		.11	.0121
O	14.37	14.37	0	0
	14.36		.01	.0001
P	11.85	11.85	0	0
	11.85		0	0
Q	14.79	14.73	.06	.0036
	14.70		.03	.0009
	14.70		.03	.0009
R	17.19	17.17	.02	.0004
	17.14		.03	.0009

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 6*

FACTORS USED TO ESTIMATE THE STANDARD DEVIATION FROM RANGE

Size of Sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.591
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

*Natrella, Experimental Statistics,(8) pp. 2-6.

Table 7

SAMPLE: Ohio River Water

DETERMINATION: Nitrate (Modified Brucine)

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
0.65 mg/lN	-0.07	.0049
0.68	-0.04	.0016
0.70	-0.02	.0004
0.76	+0.04	.0016
0.79	+0.07	.0049

minate error wouldbe the cause of undue dispersion.

Example: Table 3

$s = 41$ mg/l

$\overline{X} = 192$ mg/l

$$V = \frac{41}{192} \times 100$$

$V = 21\%$

D An indication of an individual's analytical precision can be obtained by his participation in an interlaboratory study. In this case, the analyst performs only one determination on each of two samples. Using Youden's Graphical Technique as an indication of an individual's precision was discussed previously in this outline in III C.

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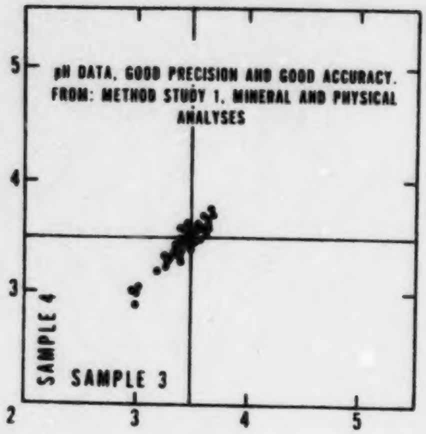
Descriptors: Accuracy, Data Collections, Data Processing, Error Analysis, Errors, Graphic Methods, Measurement, Monitoring, Precision, Quality Control

APPENDIX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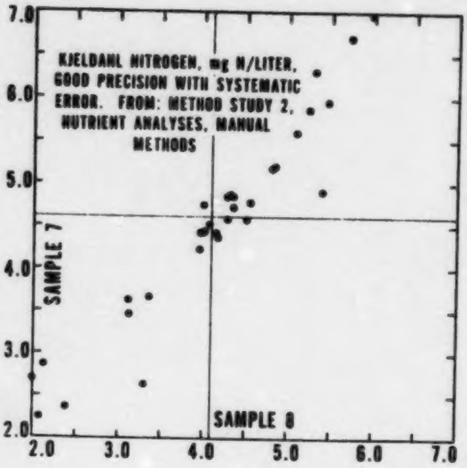
4
3
2
3
4
5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SAMPLE 8
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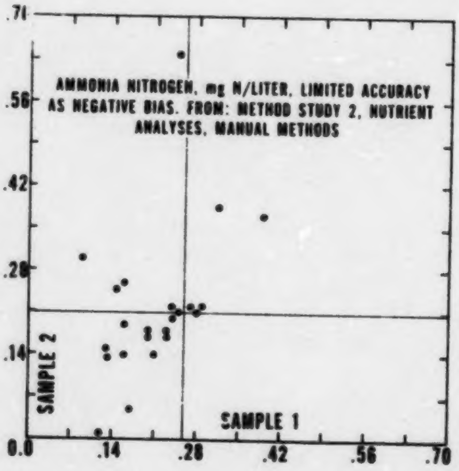
7.0


AMMONIA NITROGEN, mg N/LITER, LIMITED ACCURACY AS NEGATIVE BIAS. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS
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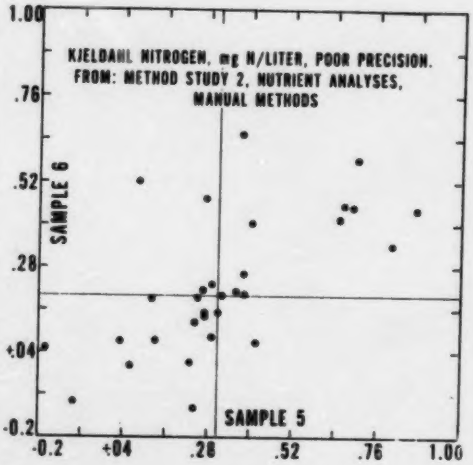
.56
.70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SAMPLE 5
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.52
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+.04
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+.04
.28
.52
.76
1.00

ELEMENTS OF A QUALITY ASSURANCE PROGRAM

I WATER QUALITY DATA

A Importance

  1 Criteria for decisions

    a Planning

    b Permit issuance

    c Compliance

    d Enforcement

    e Evaluation of treatment processes

    f Research decisions

  2 Effects of decisions

    a Social

    b Legal

    c Economic

B Requirements for Reliability

  1 Specificity

  2 Accuracy

  3 Precision

C Elements of Quality Assurance(1)

  1 Valid sample

  2 Recognized methodology

  3 Control of services, instruments, equipment and supplies

  4 Quality analytical performance

  5 Efficient data handling and reporting

D Documentation System

  1 Complete and permanent records must be kept by all field and laboratory personnel.

  2 Any procedures undertaken as quality checks should also be recorded, dated and signed.

  3 The results of any quality checks should be recorded, dated and signed.

  4 Any checks by outside service personnel should be recorded, dated, and signed.

E Quality Assurance Control Coordinator(2)

  1 Overall responsibility for program: development, implementation, administration

  2 Continuing assessment of level of operations

  3 Identification of training needs and provision to accomplish

  4 Coordinator for inter-laboratory quality control programs

II SAMPLE

A Validity(3, 4, 5, 6)

  1 Representative

  2 Properly collected

  3 Clean, appropriate containers

  4 Approved preservation measures

  5 Analytical checks on containers and preservatives

  6 Holding times observed

B Integrity[2, 7]

1 Written procedures for all aspects of sample handling

2 Field labels, records, seal

3 Appropriate transport to laboratory

4 Logging in system

5 Appropriate storage conditions and holding time

6 System for distribution for analysis

7 System for storage or discard

8 System for chain-of-custody documentation

III RECOGNIZED METHODOLOGY

A Need for Standardization

1 Within one laboratory

2 Between cooperating laboratories

3 Users of common data bank

4 Nation-wide requirements

B Criteria for Selection[6]

1 Specificity with accuracy and precision

2 Validity established by sufficient use and evaluation

3 Equipment and skill requirements normally available

4 Time requirement reasonable

C Sources

1 Annual Book of ASTM Standards[3]

2 Standard Methods for the Examination of Water and Wastewater[5]

3 Methods for Chemical Analysis of Water and Wastes[6]

4 U.S. Geological Survey Techniques of Water Resources Inventory[8]

5 Others

D Commonly-Used Types[9]

1 Various sample treatments (filtration, digestion, etc.)

2 Electrode-meters

3 General analytical methods

a Volumetric analysis

b Gravimetric procedures

c Combustion

4 Photometric methods

a Atomic absorption

b Flame emission

c Colorimetry

5 Gas chromatography

E Selection on Basis of Use of Data

1 Compliance monitoring

a National Pollutant Discharge Elimination System and State Certifications[10]

1) Use of alternate procedures

2) Procedures for non-listed parameters

b National Interim Primary Drinking Water Regulations[11]

1) Use of alternate procedures

2) Procedures for non-listed parameters

2 State monitoring programs[12]

a Fixed station ambient monitoring

b Intensive survey programs

3 Local regulations

4 Pre-survey field investigations

5 Control of treatment processes

F Using Recognized Procedures

1 Written step-by-step laboratory manuals

2 Strict adherence to reference source

3 Record of modifications and why

G Field Kits

1 Shortcomings

2 Uses

IV CONTROL OF SERVICES, INSTRUMENTS, EQUIPMENT AND SUPPLIES[1]

A Services

1 Distilled water

a Ammonia-free

b Carbon dioxide-free

c Ion-free

d Low organic background

2 Compressed air

a Dry

b Oil-free

c No contaminants

3 Electrical service

a Adequate voltage

b Constant voltage

c Appropriate grounding

d Efficient lighting

B Instruments

Applicable to laboratory and field instruments; and, as possible, fixed continuous monitoring devices.

1 Written requirements for daily warm up, standardization, calibration, and/or optimization procedures.

2 Standards available to perform daily check procedures. Some examples:

a Standardized weights

b Certified thermometer

c Filter (or solution) for wavelength alignment check

d Standard reference materials with standard absorption curves

e Standard resistor

f Calibration solutions (buffers, conductivity or turbidity standards)

g Parameter standards to establish or to check calibration curves

h Radioactive standards with date and count

3 Written trouble-shooting procedures

4 Schedule for required replacement or cleaning procedures

5 Schedule for check and/or adjustments by service personnel

C Laboratory Equipment

1 Great variety

a Of materials (glass, plastic, porcelain, etc.)

b Of grades

c Of accuracy in calibration

d Of specific properties

e Of unique construction

2 Selection depends on function

a Measurement and delivery of volumes require varying degrees of accuracy.

b Storage of reagents and solutions necessitates composition considerations such as:

1) Polyethylene bottles for solutions of boron, silica and alkali

2) Glass containers for organics

3) Brown glass for light-sensitive solutions

c Confinement of reactions may present special requirements such as:

1) Ground glass joints

2) Teflon plugs

3) Special resistance to thermal shock

4) Impervious to digestion conditions

d Volumetric analyses involve:

1) Very accurately calibrated glassware

2) Consideration of the temperature at which the apparatus was calibrated

e Other laboratory operations like filtration, ion exchange, absorption and extractions may require specialized construction like fritted ware which has pressure and thermal shock limits.

3 Cleaning procedures

a Basis of selection

1) Appropriate for the composition material

2) Appropriate for materials to be removed

3) Appropriate for subsequent use - (Avoid introducing contaminants)

b Definite program

1) Standardized, consistent, mandatory

2) Analytical checks on effectiveness

D Laboratory Supplies - Reagents, Solvents and Gases(1)

1 Required purity depends on:

a What is measured

b Sensitivity of method

c Specificity of detection system

2 General guides

If purity is not specified in the method, some general guides are:

a General inorganic analyses

1) Analytical reagent (AR) grade chemicals, except use primary standard grade for standardizing solutions.

2) Distilled water and solvents free of constituent

3) Commercial grade gases

b Metals analyses by flame

  1) Spectroquality chemicals for standards

  2) Spectroquality recommended for other reagents and solvents, though analytical reagent grade may be satisfactory.

  3) Acids should be distilled in glass

  4) Deionized distilled water

  5) Commercial grade or laboratory-supplied gases

c Radiological analyses

  1) Scintillation grade reagents and solvents

  2) High purity, extra dry gases with low radioactive background

d Organic analyses

  1) Reference grade when available, AR at minimum

  2) For gas chromatography (GC), various detectors require absence of certain classes of compounds, and may necessitate treatment of chemicals.

  3) Pesticide quality solvents For GC, check assay.

  4) Type of detector affects gas quality required. Molecular-sieve carrier-gas filters and drying tubes are required on combustion gases.

3 Program for assuring quality

a Written purity requirements according to methods utilized

b Date all on receipt.

c Observe shelf life recommendations. Dicard date on container.

d Observe appropriate storage requirements,

e Check assay for possible interferences,

f Run reagent and solvent blanks,

g As applicable, check background of reagents and solvents,

h Run method blanks (all reagents and solvents) with every series of samples or one for every nine samples,

i Definite procedures for limits of error, clean-up procedures or application of correction factors

j Replace gas cylinders at 100-200 psi.

4 Procedures for removing impurities

a Recrystallization

b Precipitation

c Distillation

d Washing with solvent(s) used in analysis

e Aging (gases)

f Others

5 Reagent and standard solutions

a Preparation

  1) Use of primary standard grade chemicals as required

  2) Careful weighing

  3) Class A volumetric glassware

  4) Appropriate quality distilled water or solvent

  5) Label listing compound(s), concentration, date of preparation or discard, preparer

  6) Very dilute standards prepared at time of use

b Standardization as appropriate

1) Use reliable primary standards,

2) Restandardize as required by stability.

c Purchased solutions

1) Should contain chemicals specified by method

2) Should be checked for accuracy

d Storage

1) Clean containers of material suitable for solution to be stored

2) Tight-fitting stoppers or caps

3) Safeguards against evaporation of solvent, adsorption of gases and water vapor, effects of light or temperature, etc.

e Signs of deterioration

1) Discoloration

2) Formation of precipitates

3) Significant change in concentration

V QUALITY ANALYTICAL PERFORMANCE(1)

A Skilled Analyst

1 Appropriate and continuing training

2 Willingness to follow specified procedures

3 Skilled in manipulation of laboratory equipment and techniques required in analyses

4 Understanding of basic principles utilized and design of any instruments s/he uses.

5 Knowledgeable and skilled in performing the analyses for which responsible

6 Precision and accuracy performance acceptable

B Establishing Analyst Precision

Applicable except for gas chromatography and radiological instrumentation.

1 Seven replicates of four samples covering the concentration range of applicability for analysis

2 Test among routine samples over two hours or more in normal operating conditions.

3 Calculate the standard deviation for each set.

4 Compare result to precision statement for method in the source of the procedure. (It may be stated as % relative standard deviation. If so, calculate analyst results in this form).

5 Individual's precision should be better than round-robin precision results.

C Establishing Analyst Accuracy

Exceptions: gas chromatography and radiological instrumentation

1 Spike set of 7 precision replicates of concentration low in applicability range to bring final to twice original.

2 Spike set of 7 precision replicates of mid-range concentration to bring final to about 75% of upper limit of applicability.

3 Test among routine samples over two hours or more in normal operating conditions.

4 Calculate % recovery for each set using average of results from the precision check and the recorded spike amounts.

5 Compare result to accuracy statement for method in the source of the procedure. (It may be stated as % bias, i.e., % recovery-100%).

6 Individual's accuracy should be better than round-robin accuracy results.

D Daily Performance Evaluation

1 At least two standards (high and low) analyzed with a blank to verify an established standard curve (comparable operating conditions).

2 Some methods require daily preparation of a standard curve.

3 One of about every 10 samples should be a duplicate to check precision according to acceptable standard deviation (or % relative std. deviation).

4 One of about every 10 samples should be a spiked sample to check accuracy according to acceptable % recovery (or % bias).

E Documentation of Daily Performance

1 After 20 sets of duplicate data results or of spiked sample results have been collected, control charts for precision and accuracy, respectively, can be constructed.

2 A variety of construction methods is available.

3 Plot succeeding results on the appropriate chart.

4 Charts document reliability of data.

5 Charts give signal of out-of-control numbers, trends toward out-of-control conditions, improved performance, etc.

F Interlaboratory Checks on Performance(2)

1 Quality Control samples for many constituents are available from EPA at no charge through EPA Regional Quality Assurance Coordinators. The concentration is provided with the sample. These might be run every three to six months.

2 Run split samples and compare results with the other laboratory.

3 Run performance samples (unknowns) available from EPA at no charge.

4 Participate in round-robin method and performance evaluation studies.

5 Participate in laboratory evaluation programs.

VI DATA HANDLING AND REPORTING(1)

A laboratory must have a program for systematic and uniform recording of data, and for processing and reporting it in proper form for interpretation and use.

A The Analytical Value

1 Correct calculation formulas reduced to simplest factors for quick, correct calculations.

2 Provisions for cross-checking calculations

3 Rounding-off rules uniformly applied

4 Significant figures established for each analysis

B Processing

1 Determine control chart approach and statistical calculations required for quality assurance and report purposes.

2 Develop report forms to provide complete data documentation and permanent records, and also to facilitate data processing.

a To avoid copying errors, the number of forms should be minimal.

C Reporting

The program for data handling should provide data in the form/units required for reporting.

D Storage

1 For some types of data, laboratory records must be kept readily available to regulatory agencies for a period of time.

2 A bound notebook or preprinted data forms permanently bound provide good documentation.

3 STORET is a system for storage and retrieval of water quality data. It is a State/Federal cooperative activity which provides States with direct access into the central computer system.

4 Many agencies have access to local systems for storage and retrieval of data.

VII SAFETY CONSIDERATIONS[13]

A Laboratory Facilities

B Emergency Equipment

C Program for Health Checks as Required

D Program for Inventory and Control of Toxic and Hazardous Materials and Test Wastes

E Safety Officer-Responsibilities

1 Information

2 Planning

3 Inspection

4 Implementation

5 Evaluation

6 Reports

VIII EPA Regional QA Coordinators

A Each of the ten EPA Regions has a Quality Assurance Coordinator.

1 Implements program in regional laboratory

2 Maintains relations and serves as source of information for state and interstate agencies within the region

3 Serves as liason for EPA's Environmental Monitoring and Support Laboratory (EMSL).

B The name, address and telephone number of the regional QA Coordinator can be obtained from the EPA Regional Administrator's Office or from EPA-EMSL, Cincinnati, Ohio 45268.

IX SUMMARY

Quality Assurance regarding water quality (or any type of) laboratory data requires planning, control and checking for every phase of the operation from sample collection through storage of the data. This outline contains a basic checklist of information and items to be considered when developing a program to facilitate quality analytical performance by laboratory personnel.

To make the program effective, procedures must be written, responsibilities must be clearly defined and assigned, and individuals must be accountable. Development and daily performance of such a program which meets the needs of an individual laboratory (or agency) will take time. Considering the importance of the data produced, the investment in assuring its reliability is a sound one.

REFERENCES

1 Handbook for Analytical Quality Control in Water and Wastewater Laboratories, 1979, U.S. EPA, EMSL, Cincinnati, Ohio 45268.

2 Minimal Requirements for a Water Quality Assurance Program, EPA-440/9-75-010, U.S. EPA Office of Water Planning and Standards, Washington, D.C. 20460.

3 Annual Book of ASTM Standards (Part 31), Water, 1980, American Society for Testing and Materials, Philadelphia, PA., 19103.

4 Handbook for Monitoring Industrial Wastewater, 1973, U.S. EPA-Technology Transfer, Cincinnati, Ohio 45268.

5 Standard Methods for the Examination of Water and Wastewater, 14th edition, 1976, APHA-AWWA-WPCF, Washington, D. C., 20036.

6 Methods for Chemical Analysis of Water and Wastes, 1979, U.S. EPA-EMSL., Cincinnati, Ohio 45268.

7 Model State Water Monitoring Program, EPA-440/9-74-002, U.S. EPA Office of Water and Hazardous Materials, Washington, D. C.

8 U.S. Geological Survey Techniques of Water Resources Inventory; Book 1, 1975; Book 5, Ch. A1, 1970; Book 5, Ch. A3, 1972; et. al.; U.S. Government Printing Office, Washington, D. C. 20402.

9 Kroner, "Methodology for Chemical Analysis of Water and Wastewater" U.S. EPA-NTOTC, Cincinnati, Ohio 45268.

10 Federal Register, "Guidelines Establishing Test Procedures for the Analysis of Pollutants", Vol. 41, No. 232, December 1, 1976, pp 52780-52786. Also, Vol. 44, No. 244, December 18, 1979, pp 75028-75052 presents proposed changes. The latter is scheduled for finalization after January, 1981.

11 Federal Register, "National Interim Primary Drinking Water Regulations," Vol. 40, No. 248, December 24, 1975, pp 59566-59574. Also, Vol. 45, No 168 August 27, 1980, pp 57332-57346, "Interim Primary Drinking Water Regulations, Amendments.'

12 Federal Register, "State and Local Assistance," Vol. 41, No. 82, April 27, 1976 pp 17694-17700.

13 Safety Management Manual, 1972, U. S. EPA, Washington, DC 20460.

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DESCRIPTORS: Analytical Techniques, Chemical Analyses, Quality Assurance, Quality Control, Reliability, Water Analysis

USE OF A SPECTROPHOTOMETER

I SCOPE AND APPLICATION

A Colorimetry

Many water quality tests depend on a treating a series of calibration standard solutions which contain known concentrations of a constituent of interest, and also the sample(s) with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color. A spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by equal "thicknesses" of the solutions. Results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on the curve to determine the corresponding concentration.

B Lambert Beer Law

States the applicable relationships:

$$A = e\ b\ c$$

1 A = absorbance

2 e = molar absorptivity

3 b = light path in cm

4 c = concentration in moles/liter

II APPARATUS

A Requirements

Are given as part of the method write-up

1 The applicable wavelength is specified. The unit used is nanometers (nm).

2 The light path (cell dimension) is often open-ended, e.g., "one cm or longer." One must know the light path length in the available spectrophotometer, because it is inversely related to the usable concentrations in the test. (Longer path lengths detect lower concentrations).

3 NOTE: For National Pollutant Discharge Elimination System (permit), or for Drinking Water Regulations test requirements, check with the issuing/report agency before using light paths (cells) that differ from the length specified in the approved method. If you have permission to use an alternate path length, concentrations for the test can be adjusted accordingly. These adjustments are discussed in IV and in VII (below).

III PREPARATION OF THE SPECTROPHOTOMETER

A Phototube/Filter

1 May have to choose a phototube for use above or below a particular wavelength.

2 A filter may be required.

3 If the available instrument involves a choice, check that the phototube (and filter, if applicable,) required for the wavelength to be used is in the instrument.

4 Always handle and wipe off the phototube and/or filter with tissue to avoid leaving fingerprints.

B Cell compartment

1 This area must be kept clean and dry at all times.

C Cells

1 A set must "match" each other in optical properties. To check this, use the same solution at the same wavelength, and verify that the absorbance value is the same for each cell.

2 Alternatively, a single cell can be used if it is thoroughly rinsed after each reading.

3 Instrument cells should be free of scratches and scrupulously clean.

  a Use detergents, organic solvents or 1:1 nitric acid-water.

  b Caustic cleaning compounds might etch the cells.

  c Dichromate solutions are not recommended because of adsorption possibilities.

  d Rinse with tap, then distilled water.

  e A final rinsing and drying with alcohol or acetone before storage is a preferred practice.

D Warm-Up

1 Plug in the power cord.

2 Turn the power switch on and give it an additional half-turn to keep the needle from "pegging."

3 Wait to use until the recommended warm-up time has passed. Anywhere from 10 to 30 minutes may be required.

4 If the instrument drifts during zeroing, allow a longer time.

E Wavelength Alignment

An excellent point is the known, maximum absorption for a dilute solution of potassium permanganate which has a dual peak at 526 nm and 546 nm. Use 2 matched cells for the following steps:

1 Prepare a dilute solution of potassium permanganate (about 10 mg/l).

2 Follow the steps in VI A, Zeroing Operation, using a wavelength of 510 nm, and distilled water as a "reagent blank." Keep the water in the cell during this entire procedure.

3 Rinse the matched cell two times with tap water, then two times with the permanganate solution.

4 Fill the cell three-fourths full with the permanganate solution. Keep the permanganate solution in this cell during this entire procedure.

5 Thoroughly wipe the cell with a tissue. Hold the cell by the top edges.

6 Open the cover and gently insert the cell, aligning it to the ridge as before.

7 Close the cover.

8 Record the wavelength and the absorbance reading on a sheet of paper.

9 Remove the cell of permanganate solution and close the cover.

10 Set the wavelength control at the next graduation (+ 5nm).

11 If the needle is not at infinite (symbol ∞) absorbance, use the left knob to re-set it.

12 Insert the cell containing distilled water using the techniques noted in 5, 6 and 7 above.

13 If necessary, use the right knob to re-set the needle at zero absorbance.

14 Remove the cell and insert the cell of permanganate solution using the techniques noted in 5, 6 and 7 above.

15 Record the wavelength and the absorbance reading.

16 Repeat steps 9 through 15 above until absorbance readings are recorded at 5 nm increments from 510 nm through 560 nm.

17 Make a graph plotting absorbance readings against wavelengths. With very good resolution, there will be two peaks - one at 525 nm and one at 545 nm. A single flat topped "peak" between these two wavelengths is acceptable.

18 If the maximum absorbances [peak(s)] occur below or above 526 nm or 546 nm, and at a number of scale units different from the stated instrument accuracy, the wavelength control is misaligned. To compensate for this until the instrument can be serviced, add or subtract the error scale units when setting wavelengths for subsequent tests.

IV CALIBRATION STANDARDS

A Requirements

A set of calibration standards is required, with concentrations usable in the available spectrophotometer cell (light path length).

1 The method reference may provide a table of light path lengths and the corresponding applicable concentration range for calibration standards, so one can choose the range required for his instrument cell or sample concentration.

2 The method reference may give directions for preparing one range of concentrations for a given light path length. If your cell provides a different length, your concentration requirements can be easily calculated by recalling that the light path length is inversely related to concentration. Thus, if your cell is twice the given path length, you need the given concentrations divided by two.

3 The method reference may give directions for preparing only one range of concentrations for the calibration standards, and then not be specific about the associated path length. You will have to test if the range is applicable to your instrument by preparing the given concentrations, obtaining absorbance values for them and checking the results according to section VII (below).

B Preparation

The calibration standards required for spectrophotometric measurements are so dilute, that they are commonly prepared by diluting stronger solutions. These are described in general terms below. Weights and volumes involved in preparing these solutions for a specific test can be found in the method write-up.

1 Stock Solutions

a Prepare by weighing or measuring a small amount of a chemical containing the constituent of interest and dissolving it to a one liter volume.

b Common stock solutions have concentrations in the range of 0.1 to 1.0 mg/ml.

c Most are refrigerated for storage and some are further treated by adding a preservative. Many are stable up to six months.

2 Standard Solutions

a Prepare by diluting a stock solution (at room temperature). Common volumes are 10.0 or 20.0 ml of stock diluted to one liter.

b Resulting standard solutions have concentrations in the range of 1.0 to 10.0 ug/ml.

c Although some standard solutions may be stable for a period of time, it is a recommended practice to prepare them on the day of use.

3 Calibration (Working) Standard Solutions

a Prepare by diluting a standard solution. Usually a set of calibration standards is required so that resulting concentrations give five to seven results within the sensitivity limits of the instrument. Common volumes are 1 to 10 ml of standard solution diluted to 100 ml.

b Resulting solutions might have concentrations in the range of 0.01 and 1.0 ug/ml.

c A reagent blank (distilled water) should be included in the set of standards.

4 Adjusting Concentrations

a You may find it necessary to adjust preparation quantities given in the method write-up, because your cell (light path length) differs from the example.

b These adjustments are discussed in A Requirements (above), and are usually applied to the Standard (intermediate) Solution.

C Chemical Treatment

1 Most colorimetric methods require that the calibration standards (including the reagent blank) are to be treated as the sample. Thus, they are to be processed through pretreatments and through the test as if they were samples. Then any test effects on sample results will be compensated by the same effects on results obtained for the treated standards.

2 One should be aware that pH is a critical condition for most colorimetric reactions. Ordinarily, a pH adjustment is included in the test method and reagents include chemicals to control pH. Thus,the processed standards correspond to the samples regarding pH, and thus they correspond in degree of color development. If standards are processed in some other manner, they must be pH adjusted to correspond to the samples at the time of color development.

V SAMPLE DILUTIONS

A Concentration Limits

The concentration of the sample must result in an absorbance within the range of the calibration standards, i.e., accurately detectable in the light path provided by the instrument. A dilution before analysis may be required to accomplish this. It is not accurate to dilute a sample after processing in order to obtain a usable absorbance reading.

1 Record dilution volumes so a dilution factor can be calculated and applied to results.

2 An analyst often has a good estimate of the expected concentration of a sample if s/he routinely tests samples from the same source. In this case, a single dilution, if any, is usually sufficient.

3 If a sample is from an unknown source, the analyst has several choices.

a Process the sample. If the reading shows it is too concentrated, dilute it until you get a value in the usable range. This result is not accurate enough to report, but you now know how to dilute the sample to process it through the test to get usable results.

b Prepare at least a 50% dilution and analyze it plus an undiluted aliquot.

c Prepare a variety of dilutions.

d Use some other analytical method to get a rough estimate of the expected concentration.

B Final Volumes

1 Dilute to a final volume sufficient to rinse the measuring glassware and provide the test volume cited in the referenced method.

2 Save any undiluted sample.

VI PROCEDURE FOR USING A SPECTROPHOTOMETER

A Zeroing Operation

The following steps have been written for spectrophotometers used in this course. Check the manual for the available instrument for the steps applicable for your work.

1 Set the wavelength control to the setting specified for the standards you are testing. Approach the setting by beginning below the number and dialing up to it.

2 If a cell is in the holder, remove it.

3 Close the cell holder cover.

4 Turn the power switch/zero control (left) knob until the needle reads infinite (symbol ∞) absorbance (on the lower scale).

5 Rinse a cell two times with tap water, two times with distilled water, then two times with the reagent blank solution.

6 Fill the cell about three-fourths full with reagent blank solution.

7 Thoroughly wipe the outside of the cell with a tissue to remove fingerprints and any spilled solution. Hold the cell by the top edges.

8 Open the cell holder cover and gently slide the cell down into the sample holder.

9 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

10 Close the cover and turn the light control (right) knob until the needle reads zero absorbance (on the lower scale).

11 Record an absorbance of zero for this zero concentration solution on a data sheet. (See next page).

12 Slowly remove the cell and close the cover. (No solution should spill inside the instrument). Keep the solution in the cell.

13 The needle should return to the infinite absorbance setting. It it does not:

a Reset the needle to the ∞ absorbance mark using the power switch/zero (left) control knob.

b Re-test the reagent blank solution using steps 7 through 12 above.

c If the needle does not return to the ∞ absorbance mark, another setting as noted in a. and b. is required. Additional warm-up time may be necessary before these settings can be made.

B Reading Absorbances

Using a single cell in the spectrophotometers used in this course

1 Discard any solution in the cell.

2 Rinse the cell two times with tap water, and two times with distilled water. Then rinse it two times with the lowest concentration standard remaining to be tested, or with processed sample.

3 Fill the cell about three-fourths full with the same standard or sample.

4 Use a tissue to remove any fingerprints from the cell and any droplets on the outside. Hold the cell by the top edges.

5 Open the cell holder cover and gently slide the cell down into the sample holder.

6 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

7 Close the cover.

8 Record the concentration of the standard and its absorbance on a data sheet. (For a sample, record its identification code and its absorbance on the data sheet).

DATA SHEET

Concentration mg/liter	Absorbance
0.00	
SAMPLE	
SAMPLE	

9 Repeat steps 1 through 8 (above) for each standard and sample to be tested. If a large number of measurements are to be made, check the instrument calibration every fifth reading.

a Use another aliquot of a solution already tested to see if the same reading is obtained. If not, repeat the zeroing operation in A (above).

b Alternatively, you can use the blank, if supply permits, and repeat the zeroing operation in A. (above).

10 When all the readings have been obtained, discard any solution remaining in the cell and rinse the cell with tap water. Clean the cell more thoroughly, (III C. 3), as soon as possible.

11 If no other tests are to be done, turn off the instrument, pull out the plug and replace any protective covering.

VII CHECKING RESULTS

A Readings Greater Than 0.70

On our instrument, these are considered to be inaccurate. Check the manual for your instrument or check the scale divisions to determine the limit for other models.

1 Do not use readings greater than 0.70 to develop a calibration curve.

2 From five to eight points (counting zero) are recommended for constructing a calibration curve. If you have fewer than five usable values, you should not draw a curve.

3 To prevent excessively high values in future tests, decrease the cell path length, if possible, by using an adapter and smaller cell.

4 If you cannot decrease the cell path length, you can at least obtain enough values to construct a curve. Prepare standards with five to eight concentrations ranging from zero to the concentration of the standard having an absorbance nearest to 0.70. This gives you more values for a curve, but it reduces the applicable range of the test. Usually the sample can be diluted before testing so the result will fit on the standard curve.

B Highest Reading is Less Than 0.6

1 Increase the cell path length by using a larger cell. A higher reading results.

2 Prepare a different set of standards with greater concentrations.

VIII CONSTRUCTING A CALIBRATION CURVE

If you have from five to eight usable absorbance values, you can construct a concentration curve.

A Graph Paper

Should be divided into squares of equal size in both directions

B Concentration Axis

1 Labeling

The longer side should be labeled at equal intervals with the concentrations of the calibration standards marked from 0.0 to at least the highest concentration recorded for the standards on the data sheet.

2 Units

a It is most convenient to express these concentrations in the units to be reported. Otherwise, a unit-conversion factor would have to be applied to obtain final, reportable values every time you use the curve.

b Example: If you dilute a standard solution to make 100.0 ml volumes of calibration standards, you have a choice in expressing the resulting concentrations. You can use weight/100 ml, or you can calculate weight/1 liter. If you are to report results as weight/liter, but you construct your curve using weight/100 ml, you will have to multiply every sample result from the curve by $\frac{1000}{100}$ or 10 to obtain the reportable value. It is much easier to convert the original calibration standard concentrations to the desired units and to use these as labels on the graph.

C Absorbance Axis

1 Labeling

The shorter side should be labeled at equal intervals with absorbance numbers marked from 0.00 to at least 0.70 absorbance units.

D Plotting the Curve

1 Use the absorbances recorded for each standard concentration to plot points for the curve.

2 The points should fall in a reasonably straight line.

3 Use a straight-edge to draw a line of best fit through the points. If the points do not all fall on the line, an acceptable result is an equal number of points falling closely above, as well as below the line. Experience provides a basis for judging acceptability.

4 It is not permissable to extrapolate the curve.

IX USING THE CALIBRATION CURVE

A Finding concentration of the sample

1 Use the absorbance value(s) recorded for the sample(s), and the calibration curve to find the concentration(s). If the concentration units differ from those required for reporting results, apply a unit conversion factor (VIIIB. 2).

2 If more than one dilution of a sample was tested, use the result that falls nearest the middle of the curve.

B If a sample was diluted, calculate the dilution factor and apply it to the concentration you find for the sample from the calibration curve.

1 Dilution Factor=

$$\frac{\text{final dilution volume}}{\text{ml sample used in dilution}}$$

2 Example: You diluted 10 ml sample to 50 ml. The concentration found by using a calibration curve was 0.5 mg/liter.

Then·

$$\text{constituent, mg/l} = \frac{50\text{ml}}{10\text{ ml}} \times \frac{0.5\text{ mg}}{\text{liter}}$$

$$= 2.5\text{ mg/liter}$$

This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Chemical Analysis, Colorimetry, Laboratory Tests, Spectrophotometry

DISSOLVED OXYGEN
Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G Minimum allowable DO concentrations are specified in all Water Quality standards.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

   a Resulting benthic deposits continue to respire as bed loads.

   b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

   c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

   d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

   e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

   f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

   g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia.

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, and revised by Charles R. Feldmann, Chemist, National Training & Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

DISSOLVED OXYGEN

Determination by the Winkler Iodometric Titration - Azide Modification

This method is applicable for use with most wastewaters and streams that contain nitrate nitrogen and not more than 1 mg/1 of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 ml of fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/1 ferric iron.

The azide modification is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution, or which are oxidized by free iodine in an acid solution; (d) untreated domestic sewage; (e) biological flocs; and (f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4 \qquad (a)$$

$$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2 \qquad (b)$$

$$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \qquad (c)$$

$$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \qquad (d)$$

$$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI \qquad (e)$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross

/

excess of reagents is used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the floc to settle until there is clear liquid above the floc, repeating the inversion, & allowing the floc to settle about two-thirds of the way down in the bottle. The reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle until there is clear liquid above the floc, repeat the inversion, & allow the floc to settle about two-thirds of the way down in the bottle. Highly saline & other test waters may settle very slowly. In this case, allow some reasonable time (e.g. 2 min.) for completion of the reaction.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 normal thiosulfate. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality; e.g.,

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl or H_2O_2 may raise the DO titration while H_2S, & SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^{=}$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$\begin{array}{ll} 2HNO_2 + 2\ HI \rightarrow I_2 + 4H_2O + N_2O_2 & (a) \\ \uparrow \qquad\qquad\qquad\qquad\qquad\qquad \downarrow & \\ 2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2 & (b) \end{array}$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of NO_2^- N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe+++ may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U. S. Environmental Protection Agency, Environment Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, and revised by C. R. Feldmann, Chemist, National Training & Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Dissolved Oxygen, Oxygen, Water Analysis

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN
Winkler Method-Azide Modification

I APPLICABILITY

A The azide modification is used for most wastewaters and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/l. If 1 ml 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg ferric iron/l.

B Reducing and oxidizing materials should be absent.

C Other materials which interfere with the azide modification are: sulfite, thiosulfate, appreciable quantities of free chlorine or hypochlorite, high suspended solids, organic substances readily oxidized in a highly alkaline medium, organic substances readily oxidized by iodine in an acid medium, untreated domestic sewage, biological flocs, and color which may interfere with endpoint detection. A dissolved oxygen meter should be used when these materials are present in the sample.

II REAGENTS

Distilled water is to be used for the preparation of all solutions.

A Manganous Sulfate Solution

Dissolve 480 g $MnSO_4 \cdot 4H_2O$ (or 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$) in water and dilute to 1 liter.

B Alkaline-Iodide-Azide Solution

Dissolve 500 g sodium hydroxide (or 700 g potassium hydroxide) and 135 g sodium iodide (or 150 g potassium iodide) in water and dilute to 1 liter. To this solution add 10 g of sodium azide dissolved in 40 ml water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a refrigerator at 10°C.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform.

G Potassium Biiodate Solution 0.0375N

Dry about 5 g of $KH(IO_3)_2$ at 103°C for two hours and cool in a desiccator. Dissolve 4.873 g of the solid in water and dilute to 1 liter. Dilute 250 ml of this solution to 1 liter.

H Sulfuric Acid Solution 10%

Add 10 ml of conc sulfuric acid to 90 ml of water. Mix thoroughly and cool.

I Potassium Iodide Crystals

III STANDARDIZATION OF THE TITRANT

A Dissolve 1-3 g of potassium iodide in 100-150 ml of water.

B Add 10 ml of 10% sulfuric acid and mix.

C Pipet in 20 ml of the 0.0375N potassium biiodate and mix. Place in the dark for 5 minutes.

D Titrate with the 0.0375N sodium thiosulfate standard titrant to the appearance of a pale yellow color.

Mix the solution thoroughly during the titration.

E Add 1-2 ml of starch solution and mix. The solution is now blue in color.

F Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

G Record the ml of titrant used.

H Calculate the N of the sodium thiosulfate standard titrant. It will be approximately 0.0375.

$$N = \frac{\text{(ml x N) of the biiodate}}{\text{ml of titrant}}$$

$$= \frac{20.0 \times 0.0375}{\text{ml of titrant}}$$

$$= \frac{0.75}{\text{ml of titrant}}$$

IV PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkaline iodide-azide

To a full BOD bottle (300 ml ± 3 ml), add 2 ml manganous sulfate solution and 2 ml alkaline-iodide azide reagent with the tip of each pipette below the surface of the liquid.

2 Stopper the bottle without causing formation of an air bubble.

3 Rinse under running water.

4 Mix well by inverting 4-5 times.

5 Allow the precipitate to settle until at least 200 ml of clear supernate have been produced.

6 Repeat steps 4 and 5.

7 Add 2 ml conc. sulfuric acid with the tip of the pipette above the surface of the liquid.

8 Stopper the bottle without causing formation of an air bubble.

9 Rinse under running water.

10 Mix by inverting several times to dissolve the precipitate.

11 Pour contents of bottle into a wide-mouth 500 ml Erlenmeyer flask.

B TITRATION

1 Titrate with 0.0375N thiosulfate to a pale yellow color.

2 Add 1-2 ml starch solution and mix.

3 Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

4 Record the ml of titrant used.

C CALCULATION

$$\text{mg DO/l} = \frac{\text{ml titrant x N titrant x 8 x 1000}}{\text{ml sample}}$$

If the N of the titrant exactly = 0.0375,

$$\text{mg DO/l} = \frac{\text{ml titrant x 0.0375 x 8 x 1000}}{300}$$

$$= \text{ml titrant x 1}$$

$$= \text{ml titrant}$$

REFERENCE
Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Chemical Analysis, Dissolved Oxygen, Laboratory Tests, Oxygen, Water Analysis

DATA SHEET

ml of titrant = ____________

N of titrant = ____________

ml of sample = ____________

$$\text{mg DO/liter} = \frac{\text{ml titrant} \times \text{N titrant} \times 8 \times 1000}{\text{ml sample}}$$

$$= \frac{\_\_\_\_ \times \_\_\_\_ \times 8 \times 1000}{\_\_\_\_\_\_\_\_\_\_}$$

$$= \frac{\_\_\_\_}{\_\_\_\_}$$

$$=$$

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^{-}$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^{o} - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

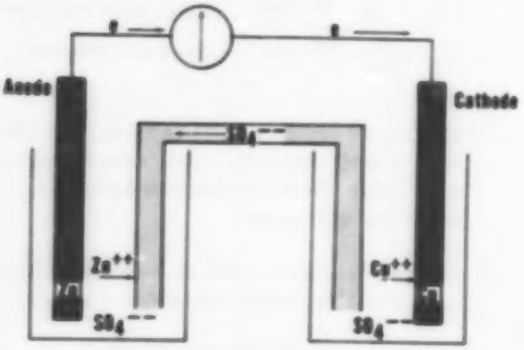
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

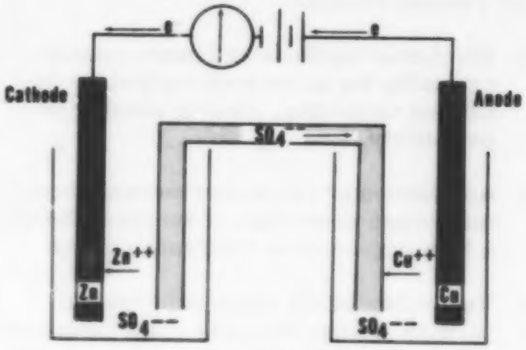
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KC1 and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

a Variable line voltage or low batteries in amplifier power circuits.

b Substandard or unsteady amplifier or resistor components.

c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

d Inadequately shielded electronic components.

e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply USEPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

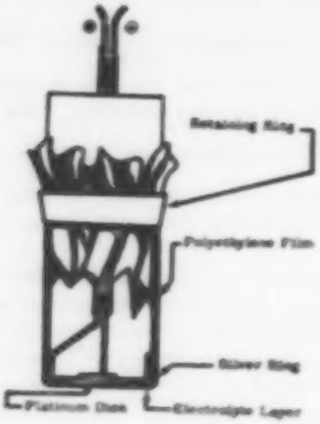

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disc
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

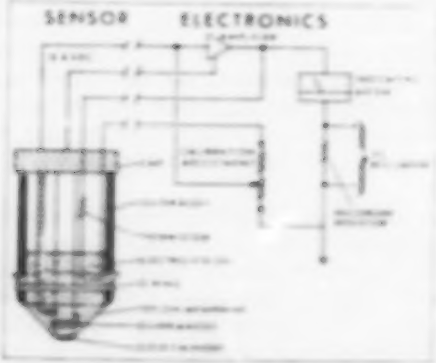

SENSOR
ELECTRONICS

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

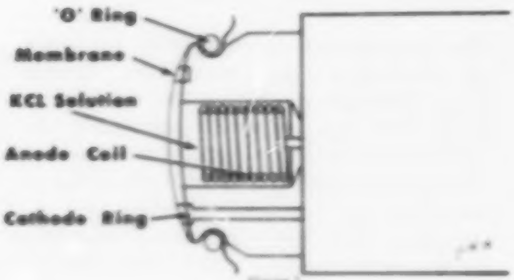
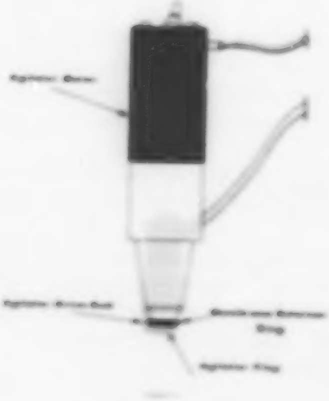

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring

Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe



E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

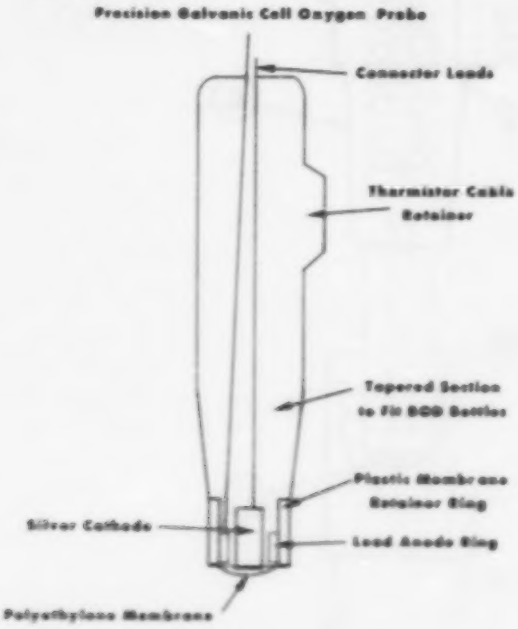

Precision Galvanic Cell Oxygen Probe
Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Figure 7

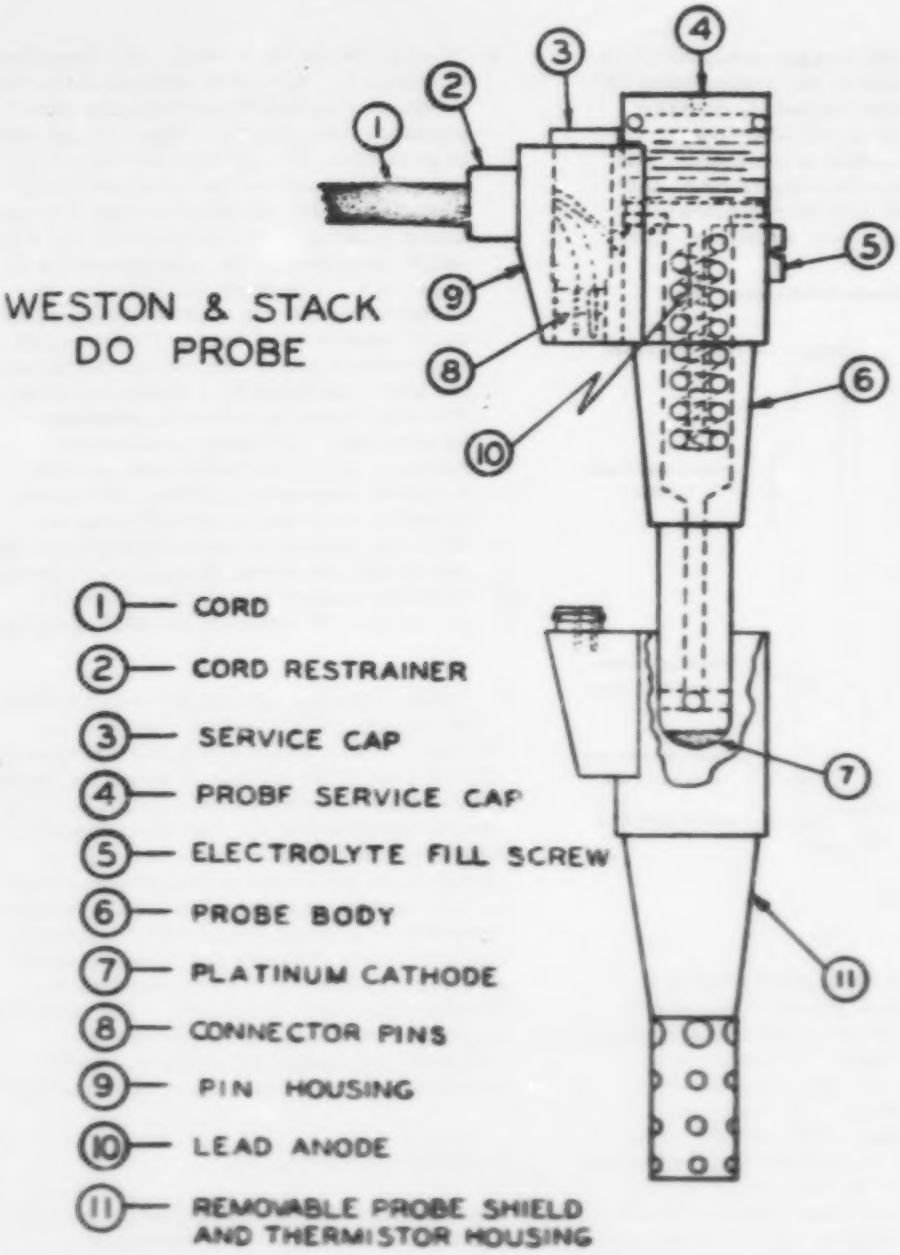
F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

This outline contains certain materials from previous outlines by D. G. Ballinger, N.C. Mikof, and J.W. Mandia. Additional information was provided by C.R. Hirth, C.N. Shadix, D.F. Krawczyk, J. Woods, and others.


WESTON & STACK
DO PROBE
①
②
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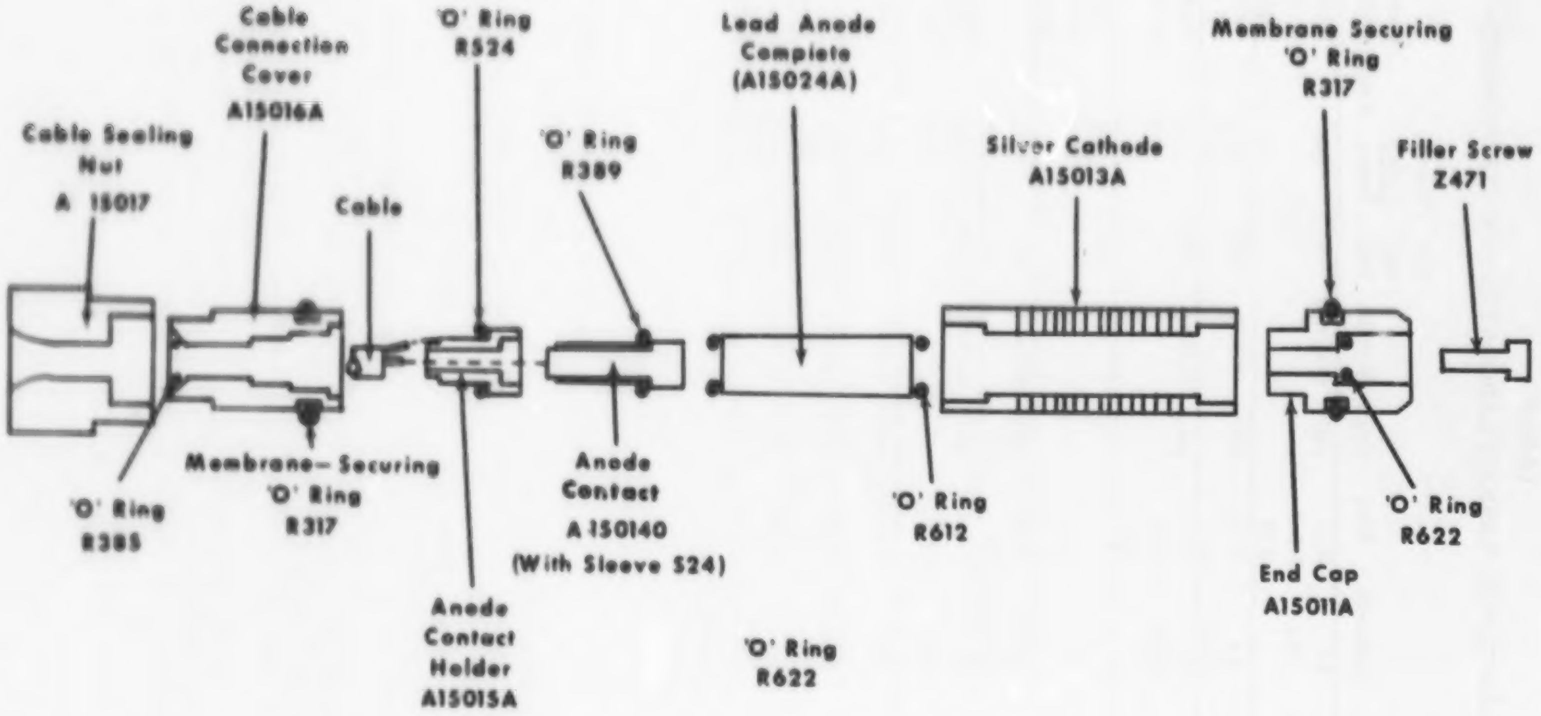
④
⑤
⑥
⑦
⑧
⑨
⑩
⑪
① CORD
② CORD RESTRAINER
③ SERVICE CAP
④ PROBF SERVICE CAP
⑤ ELECTROLYTE FILL SCREW
⑥ PROBE BODY
⑦ PLATINUM CATHODE
⑧ CONNECTOR PINS
⑨ PIN HOUSING
⑩ LEAD ANODE
⑪ REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut A 15017
Cable Connection Cover A15016A
Cable
'O' Ring R524
'O' Ring R389
Lead Anode Complete (A15024A)
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
Filler Screw Z471
'O' Ring R385
Membrane– Securing 'O' Ring R317
Anode Contact Holder A15015A
Anode Contact A 150140 (With Sleeve S24)
'O' Ring R612
End Cap A15011A
'O' Ring R622
'O' Ring R622

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22

Figure 9

TABLE 1
CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no* yes	field and bottle probe
Yellow Springs 54	″	″	″	″	″	yes	yes yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)
**Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, OH 45268 and Nate Malof, Chemist, USEPA, OWPO, National Field Investigations Center, Cincinnati, OH

Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis, Analysis, Wastewater, Oxygen

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD_5 is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD_5 is "a" fraction of total oxygen requirements.

A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.

1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.

2 This outline and Standard Methods(1) discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual(2).

B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days.

C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time. The problem is to define the shape of the deoxygenation pattern and its limits. A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD.

D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior.

II DIRECT METHOD

A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/1 and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination. General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are:

For a 5-day BOD of
5-20 mg/1, use 25 to 100% sample

For a BOD of
20-100 mg/1, use 5 to 25% sample

For a BOD of
100-500 mg/1, use 1 to 5% sample

For a BOD of
500-5000 mg/1, use 0.1 to 1.0% sample

III PROCEDURES

A Cylinder Dilution Technique

1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/l and a residual of 1 mg/l are most reliable. At least three dilutions in duplicate should be used for an unknown sample.

2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste. Fill to the one liter mark with dilution water. Carefully mix. The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.

3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing.

4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".

5 Incubate the two remaining bottles at 20°C in complete darkness. The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.

6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) above, and the bottle filled with dilution water to make the desired sample concentration for incubation. In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.

2 Continue the procedure as in A-4, 5, and 6 above.

C Seeded Cylinder Dilution Technique

1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity. Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test. Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data. Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant.

2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.

3 Estimate the sample concentration desired in accordance with A-1 and C-2 above and add the sample aliquot to the dilution cylinder.

4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms.

5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water. Mix the combined sample, seed and dilution water without excessive air entrainment.

6 Continue as in III-A steps 4, 5, and 6 above.

IV INTERPRETATION OF RESULTS

Standard Methods[(1)] includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a "________if you do, ________if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history. Saprophytes resulting in surface water stabilization may be a small fraction of the population. Re-actable oxygen-demanding components produce excessive demands upon test oxygen resources.

2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeding. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

A The DO test precision often has been used to suggest precision of the BOD result.

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0.10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies.

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water. It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample. The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common container when the residual DO reaches about 1.0 mg/1. After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test. If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs.

B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals. When the DO concentration in the smaller bottles reaches 1.0 mg/1, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary.

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container. See diagram.

C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/1 instead of 9 mg/1. Limited results are available hence the analyst must verify his technique. The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/1 of oxygen content is the top of the practical working concentration. There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression.

D Reaeration or Oxygenation Advantages and Limitations.

1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/1

2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.

3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination.

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD_5 with electronic DO probes.

A It is the responsibility of the analyst to evaluate:

1 Applicability of the specified technique and sample.

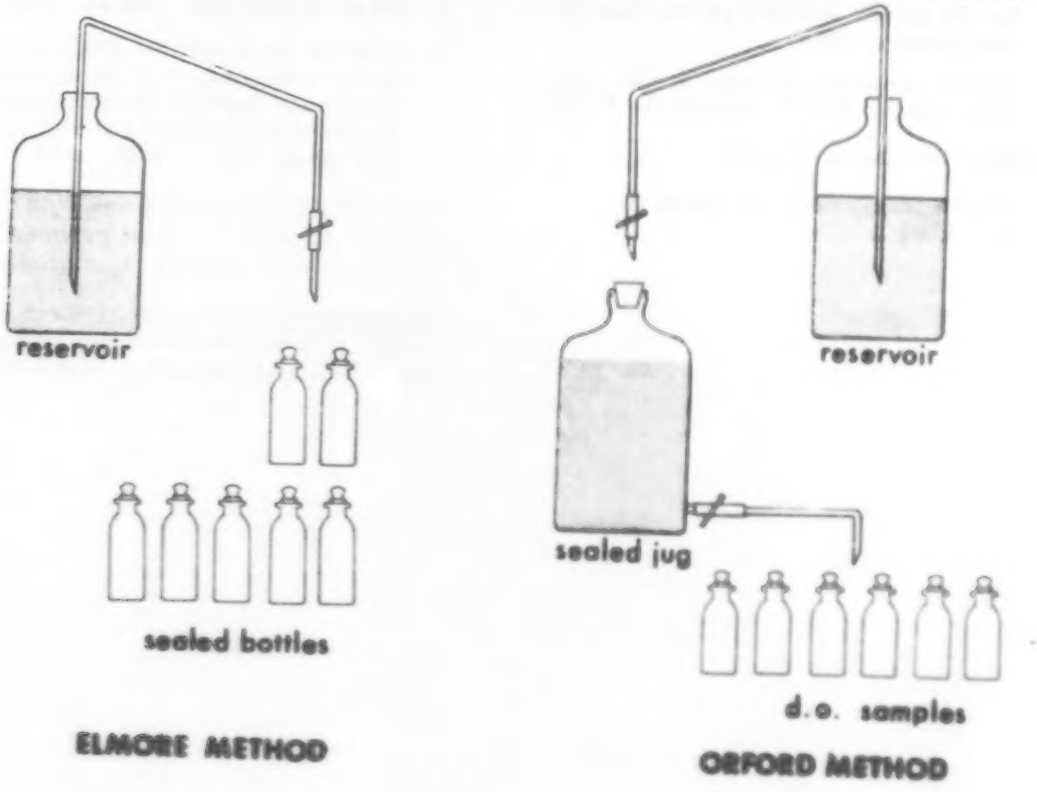
2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.

3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as ferrous iron, sulfite and sulfide which are susceptible to high rate chemical oxidation.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD

ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique. Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.

2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion.

3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after the arbitrarily selected time of 15 minutes.

4 Any difference between the calculated initial DO as obtained in 2 above, and the DO determined in 3 above, may be designated as IDOD.

5 Sample aeration, DO interference, and other factors affect results for IDOD.

C Sample Calculation of IDOD

1 Sample DO checked and shown to be 0.0 mg/l

Dilution water DO found to be 8.2 mg/l

Assume a mixture of 9 parts of dilution water and 1 part (V/V) of sample.

Calculated DO =

$1 \times 0 = 0$

$9 \times 8.2 = 73.8$

10 parts of the mixture contain 73.8/10 or 7.4 mg DO/l. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

2 The mixture described above was held for 15 minutes and the DO determined was 4.3 mg/l.

$$\text{IDOD} = \text{DO}_{\text{calc}} - \text{DO}_{\text{detm}} \times \frac{100}{\%\ \text{sample used}}$$

$$= 7.4 - 4.3 \times 10$$

$$= 31 \text{ mg IDOD/l}$$

REFERENCES

1 Standard Methods, 14th ed, 1975.

2 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio

Descriptors: Biochemical Oxygen Demand, Chemical Analysis, Dissolved Oxygen, Water Analysis, Analysis, Wastewater

BIOCHEMICAL OXYGEN DEMAND TEST
DILUTION TECHNIQUE

I GENERAL

A Standard Methods (1) lists three ways of diluting biochemical oxygen demand (BOD) samples: in a 1 or 2 liter graduated cylinder, in a bottle of known capacity (e.g., the BOD bottle), or in a volumetric flask for dilutions greater than 1: 100, followed by final dilution in the incubation bottle.

B The dissolved oxygen (DO) determinations may be made using the azide modification of the Winkler procedure, or a DO meter.

II REAGENTS

A Distilled water - obtained from a block tin or all glass still; or use deionized water. It must contain no more than 0.01 mg of copper/liter. It must be free of chlorine, chloramines, caustic alkalinity, organic material and acids. Aerate the water in one of three ways: loosely plug the container with cotton and store at 20°C for about 48 hours; shake 20°C water in a partially filled container; bubble clean compressed through 20°C water. Use distilled (but not necessarily aerated) water for the preparation of all solutions.

B Phosphate Buffer Solution - dissolve 8.5g potassium dihydrogen phosphate, KH_2PO_4, 21.75g dipotasium hydrogen phosphate, K_2HPO_4, 33.4g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$, and 1.7g ammonium chloride, NH_4Cl, in about 500 ml of water and dilute to 1 liter. The pH of this solution is 7.2. Discard it if any biological growth appears in the bottle.

C Magnesium Sulfate Solution - dissolve 22.5g magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$, in water and dilute to 1 liter.

D Calcium Chloride Solution - dissolve 27.5g anhydrous calcium chloride, $CaCl_2$, in water and dilute to 1 liter.

E Ferric Chloride Solution - dissolve 0.25g ferric chloride, $FeCl_3$, in water and dilute to 1 liter.

F Dilution water - add 1 ml each, of solutions II B, II C, II D, and II E for each liter of distilled water (IIA). If the dilution water is to be stored, add the phosphate buffer (IIB) just before use.

G Seeded Dilution Water - the standard seed material is the supernatant liquid from domestic wastewater which has been allowed to settle for 24-36 hours at 20°C. Use an amount which will produce a seed correction of at least 0.6 mg/liter. Add the seed to the dilution water (II F) on the day the dilution water is to be used.

H Sodium Sulfite Solution, 0.025N - dissolve 1.575g anhydrous sodium sulfite, Na_2SO_3, in water and dilute to 1 liter. Prepare this solution daily; it is unstable.

I Acetic Acid Solution 50% - slowly pour 50 ml acetic acid, $HC_2H_3O_2$, into 50 ml of water.

J Potassium Iodide Solution, 10% - dissolve 10g potassium iodide, KI, in 90 ml water.

K Sodium Hydroxide Solution, 1N -dissolve 4g sodium hydroxide, NaOH, in water and dilute to 100 ml.

L Sulfuric Acid Solution, 1N - slowly pour 2.8 ml of conc. sulfuric acid, H_2SO_4, into 98 ml of water.
Caution: heat will be generated.

M Powdered Starch Indicator - Thyodene is one brand name.

N Bromthymol Blue Indicator - or a pH meter.

III INTERFERENCES/PRETREATMENT

A Caustic Alkalinity or Acidity - this must be neutralized to a pH of about 7 with 1 N sulfuric acid or sodium hydroxide. Use a pH meter or bromthymol blue as an external indicator.

B Residual Chlorine Compounds - some residual chlorine will dissipate if the sample is allowed to stand for 1 or 2 hours. Higher residuals must be determined, and then neutralized. To a known volume of sample between 100 and 1000 ml, add 10 ml of acetic acid solution, 10 ml of potassium iodide solution, mix, and titrate to the disappearance of blue color with 0.025N sodium sulfite and using powdered starch indicator (or starch solution). Use a proportionate amount of the 0.025N sodium sulfite to dechlorinate the entire sample. (The portion of sample used above to determine the chlorine content of the sample should be discarded, and is not to be used for the BOD determination.) After 10-20 minutes, check a portion of the dechlorinated sample to make sure the dechlorination is complete.

C Other Toxic Substances - samples containing other toxic substances, e.g. metals in plating wastes, require special study and treatment.

D Supersaturation - if you suspect that the sample contains more than 9 mg of oxygen/liter at 20°C, shake it vigorously in a large bottle or flask, or pass clean compressed air through the sample.

IV SUGGESTED SAMPLE DILUTIONS

Standard Methods (1) suggests the following sample dilutions. However, actual dilutions should be determined on the basis of experience, or information supplied with the sample.

Type of Waste	% Dilution
Strong Trade	0.1 - 1.0
Raw & Settled Sewage	1 - 5
Oxidized Effluents	5 - 25
Polluted River Waters	25 - 100

During the 5-day incubation period, at least 2 mg of oxygen/liter must be consumed, and at least 1 mg of oxygen/liter must remain at the end of the incubation period.

V PROCEDURE

The steps below represent one of several ways in which the BOD can be set up. For example purposes, assume the dilution water does not have to be seeded.

A Siphon 20°C high quality distilled water to the 1000 ml line in a graduated cylinder. Tilt the cylinder slightly and allow the water to run down the sides of the cylinder. If the siphon was "primed", with other water, "waste" about 100 ml before filling the cylinder.

B Add 1 ml of the calcium solution and mix with a plunger-type mixer.

C Add 1 ml of the magnesium solution and mix with a plunger-type mixer.

D Add 1 ml of the ferric solution and mix with a plunger-type mixer.

E Add 1 ml of the buffer solution and mix with a plunger-type mixer. (If the dilution water were to be seeded, it would be done at this point).

F Siphon about 250 ml of the dilution water into a 1 liter graduated cylinder. If more than 750 ml of sample are to be used, less than 250 ml of dilution water would, of course, be siphoned in initially. Use the same technique as in A above.

G Measure the amount of well mixed sample to be used. Use a graduated pipet for smaller sample volumes. If solids are present in the sample, the tip of the pipet may be cut off below the bottom graduation line. For larger sample volumes, use the appropriate size graduated cylinder.

H Add the sample to the cylinder containing the 250 ml of water. Allow the sample to run down the sides of the cylinder.

I Siphon in additional dilution water to the 1000 ml line, and mix with a plunger-type mixer. If other dilutions of the same sample, or other smaples, are being set up, be sure to rinse the mixer between uses.

J Siphon the dilution water-sample mixture into two BOD bottles. Hold the end of the siphon close to the bottom of the bottle, open the siphon slowly, and keep the tip of the siphon just above the surface of the surface of the liquid as the bottle fills. Allow a small amount of the mixture to overflow the bottle. If the siphon was "primed", "waste" about 100 ml before filling the bottles.

K Insert the stoppers into the BOD bottles with a slight twisting motion. Do not use so much force that an air bubble is created.

L Determine the initial DO (DOi) on one of the bottles within 15 minutes. Use the Winkler procedure, azide modification, or a DO meter.

M Water-seal the second bottle and incubate in the dark, at 20°C ± 1°C, for five days.

N Determine the final DO (DOf) on the second bottle. Use the same method as in L above. (Recall the restrictions noted at the end of section IV).

VI EXAMPLE CALCULATIONS

DO initial = DO i = 7.5 mg/l
DO final = DO f = 2.5 mg/l
100 ml = sample volume diluted in the 1 liter graduated cylinder = 10% dilution (0.1 as a decimal fraction)

$$\text{mg five-day BOD/l} = \frac{\text{DOi - DOf}}{\text{\% sample dilution expressed as a decimal}}$$

$$\text{mg five-day BOD/l} = \frac{7.5 - 2.5}{0.1}$$

$$= 50$$

VII SEED CORRECTION

A If you do seed the dilution water, a correction must be applied to the calculation in VI above.

B Do this by setting up another five-day BOD exactly as described above, except, use seed material instead of sample.

C In this case however, the five-day oxygen depletion must be 40-70%. (In the case of the sample it was a depletion of at least 2 mg/l with at least 1 mg/l remaining). Consequently, it may be necessary to set up several dilutions of the seed in order to get one with a 40-70% depletion.

D Example Seed Correction Calculation

Two hundred fifty ml of seed material are diluted to 1000 ml with dilution water.

$$\frac{250}{1000} \times 100 = 25\% \text{ seed material}$$

DO i = 7.0 mg/l
DO f = 3.0 mg/l

Depletion = 7.0 mg/l - 3.0 mg/l
= 4.0 mg/l

$$\text{\% depletion} = \frac{4.0 \text{ mg/l}}{7.0 \text{ mg/l}} \times 100$$

$$= 56$$

Since the 25% seed dilution gave an oxygen depletion in the desired 40-70% range (56%), it can be used to calculate the seed correction.

E Example Seed Correction Calculation (Continued)

Assume that in preparing the dilution water (V A through V E), you added 2 ml of seed material to the graduated cylinder before adding dilution water to the 1000 ml line.

$\frac{2}{1000}$ x 100 = 0.2% seed material in the dilution water

F Example Seed Correction Calculation (Continued)

In the example calculation in VI, a 10% sample dilution was assumed.

If the BOD bottles contained 10% sample, they therefore contained 90% dilution water.

300 ml (volume of BOD bottles)
0.90 (% dilution water in the BOD bottles expressed as a decimal)
270.00 ml (volume of dilution water in the BOD bottles)

G Example Seed Correction Calculation (Continued)

270 ml (volume of dilution water in the BOD bottles)
0.002 (% seed material in the dilution water expressed as a decimal)
0.540 ml (volume of seed material in the BOD bottles)

H Example Seed Correction Calculation (Continued)

$\frac{0.54}{300}$ x 100 = 0.18% seed material in the BOD bottles

I You now have all the data you need to calculate the seed correction.

mg five-day BOD/l =

$$\frac{(\text{DOi} - \text{DOf}) \text{ of sample} - [(\text{DOi} - \text{DOf}) \text{ of seed material} \times \text{factor}]}{\%\text{ of sample expressed as a decimal}}$$

DOi of sample = 7.5 mg/l (from VI above)

DOf of sample = 2.5 mg/l (from VI above)

DOi of seed material = 7.0 mg/l (from VII D above)

DOf of seed material = 3.0 mg/l (from VII D above)

% of seed in the sample BOD bottles = 0.18 (from VII H above)

% of seed in the seed BOD bottles = 25 (from VII D above)

% of sample expressed as a decimal fraction = 0.1 (from VI above)

$$\text{factor} = \frac{\%\text{ of seed in the sample BOD bottles}}{\%\text{ of seed in the seed BOD bottles}}$$

$$= \frac{0.18}{25}$$

$$= 0.0072$$

Finally, mg five-day BOD/l = (7.5 - 2.5) - [(7.0 - 3.0) x 0.0072]

$$= \frac{5.0 - [4.0 \times 0.0072]}{0.1}$$

$$= \frac{5.0 - 0.03}{0.1}$$

$$= 49.7$$

VIII Dilution Water Check

A five-day BOD on unseeded dilution water must not be greater than 0.2 mg/l (and preferably not more than 0.1 mg/l.) If it is greater than 0.2 mg/l, check for contamination in the distilled water and, or, dirty BOD bottles. Do not use the value as a correction on the BOD.

REFERENCES

1 Standard Methods for the Examination of Wastewater, 14th ed, APHA, AWWA, WPCF, New York, pg 543, 1975.

This outline was prepared by Charles R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Biochemical Oxygen Demand, Chemical Analysis, Laboratory Tests, Water Analysis

DATA SHEET

Initial DO in mg/l, DOi = ______________

Final DO in mg/l. DOf = ______________

% sample dilution expressed
as a decimal (e.g., 18% = 0.18) = ______________

mg 5-day unseeded BOD in mg/liter = $\frac{DOi - DOf}{decimal}$

= $\frac{\quad - \quad}{\rule{5em}{0.4pt}}$

= ______________

SOURCES AND ANALYSIS OF ORGANIC NITROGEN

I INTRODUCTION

A Organic nitrogen refers to the nitrogen in combination with any organic radical. For sanitary engineering the main interest is the nitrogen contained in proteins, peptides, amines, amino acids, amides and related compounds of animal or vegetable origin.

II SOURCES OF ORGANIC NITROGEN

A Natural Origin

1 Dead animal and plant residues

2 Animal wastes - urea, feces

3 Autotrophic organisms - algae, s. bact.

4 Heterotrophic organisms

B Industrial Origin

1 Food processing wastewater - meat, milk, vegetables

2 Pharmaceutical wastes, antibiologicals

3 Plastics - polyamides, nitriles

4 Chemical intermediates or products

5 Dye industry - azo, nitro

III TOTAL KJELDAHL NITROGEN PROCEDURE

A Organic nitrogen is determined using the Total Kjeldahl Nitrogen (TKN) method. This determination includes both organic nitrogen and free ammonia. By distilling the free ammonia off the sample before the determination, organic nitrogen can be determined directly.

B Scope

The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but it may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines.

C Method Summary(1)

The sample is heated in the presence of a concentrated sulfuric acid-potassium sulfate-mercuric sulfate mixture and evaporated until sulfur trioxide fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled and diluted, then treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled off and then determined either by nesslerization or by titration.

1 Nesslerization (colorimetric) is the method used when the ammonia-nitrogen concentration is less than 1.0 mg N/liter.

2 For ammonia-nitrogen concentrations above 1.0 mg N/liter, the ammonia is determined by titration with 0.02 N H_2SO_4 in the presence of a mixed indicator.

For a detailed description of the procedure and reagent preparation, consult the EPA methods manual.

D Precision and Accuracy

1 Thirty-one analysts in 20 laboratories(1) used the Total Kjeldahl Nitrogen procedure to analyze natural water samples containing the following increments of organic nitrogen: 0.20, 0.31, 4.10 and 4.61 mg N/liter.

a Precision results for a standard deviation were 0.197, 0.247, 1.056 and 1.191 mg N/liter, respectively.

b Accuracy as bias was +0.03, +0.02, +0.04 and -0.08 mg N/liter, respectively.

2 The nature and composition of extraneous materials affect analytical recovery. High salt concentrations may raise digestion temperature. High concentrations of organic sample components may require excessive acid during digestion tending to low nitrogen yield.

3 The digestion temperature is critical. 380 to 390°C usually gives high analytical recovery on the more refractory nitrogen compounds of natural origin. Nitrogen losses occur above 420°C.

E Optimum temperature is associated with a digestion mix containing 1 gram of potassium sulfate for each ml of sulfuric acid. However, the digestion mixture usually contains a fraction of H_2SO_4 greater than this.

During heating, the water boils off first, followed by fuming as the H_2SO_4 becomes concentrated. Fuming and oxidation of the sample components lead to loss of H_2SO_4 and a progressive decrease in acid content during heating. As the acid decreases, the temperature of the heated mixture rises.

Valid nitrogen determinations require a slight excess of acid to retain NH_3 as NH_4HSO_4 rather than the more volatile $(NH_4)_2SO_4$. Concurrently, excess acid will tend toward incomplete oxidation of sample components as a result of lowering digestion temperature.

IV AUTOMATED PHENATE METHOD

The EPA manual(1) also presents an automated (phenate) method for organic nitrogen. The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst, then neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

V PRESERVATION OF SAMPLES

A Most nitrogen compounds are characterized by rapid conversion from one form to another by biological and chemical action. Hydrolysis, deamination, peptide formation, and other reactions may appreciably alter the original form of sample nitrogen within a short time. Organic nitrogen tends to hydrolyze to free NH_3 during storage. Therefore, valid results require prompt analysis.

B Addition of 2ml of concentrated sulfuric acid or 40 mg $HgCl_2$/liter of sample and storage at 4°C are preservation measures for samples. Even when so preserved, samples are unstable and should be analyzed as soon as possible.

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environment Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by Audrey E. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Chemical Analysis, Nitrogen, Nutrients, Water Analysis, Water Pollution Sources

AMMONIA, NITRITES AND NITRATES

I SOURCES AND SIGNIFICANCE OF AMMONIA, NITRITES AND NITRATES IN WATER

The natural occurrence of nitrogen compounds is best demonstrated by the nitrogen cycle (Figure 1).

A Ammonia

1 Occurrence

Ammonia is a product of the microbiological decay of animal and plant protein. In turn, it can be used directly to produce plant protein. Many fertilizers contain ammonia.

2 Significance

The presence of ammonia nitrogen in raw surface waters might indicate domestic pollution. Its presence in waters used for drinking purposes may require the addition of large amounts of chlorine in order to produce a free chlorine residual. The chlorine will first react with ammonia to form chloramines before it exerts its full bactericidal effects (free chlorine residual).

B Nitrites

1 Occurrence

Nitrite nitrogen occurs in water as an intermediate stage in the biological decomposition of organic nitrogen. Nitrite formers (nitrosomonas) convert ammonia under aerobic conditions to nitrites. The bacterial reduction of nitrates can also produce nitrites under anaerobic conditions. Nitrite is used as a corrosion inhibitor in industrial process water.

2 Significance

Nitrites are usually not found in surface water to a great extent. The presence of large quantities indicates a source of wastewater pollution.

C Nitrates

1 Occurrence

Nitrate formers convert nitrites under aerobic conditions to nitrates (nitrobacter). During electrical storms, large amounts of nitrogen (N_2) are oxidized to form nitrates. Finally, nitrates can be found in fertilizers.

2 Significance

Nitrates in water usually indicate the final stages of biological stabilization. Nitrate rich effluents discharging into receiving waters can, under proper environmental conditions, cause stress to stream quality by producing algal blooms. Drinking water containing excessive amounts of nitrates can cause infant methemoglobinemia.

II PRESERVATION OF AMMONIA, NITRATE AND NITRITE SAMPLES (8)

A If the sample is to be analyzed for Ammonia, Nitrate or Nitrite, cool to 4°C and analyze within 24 hours.

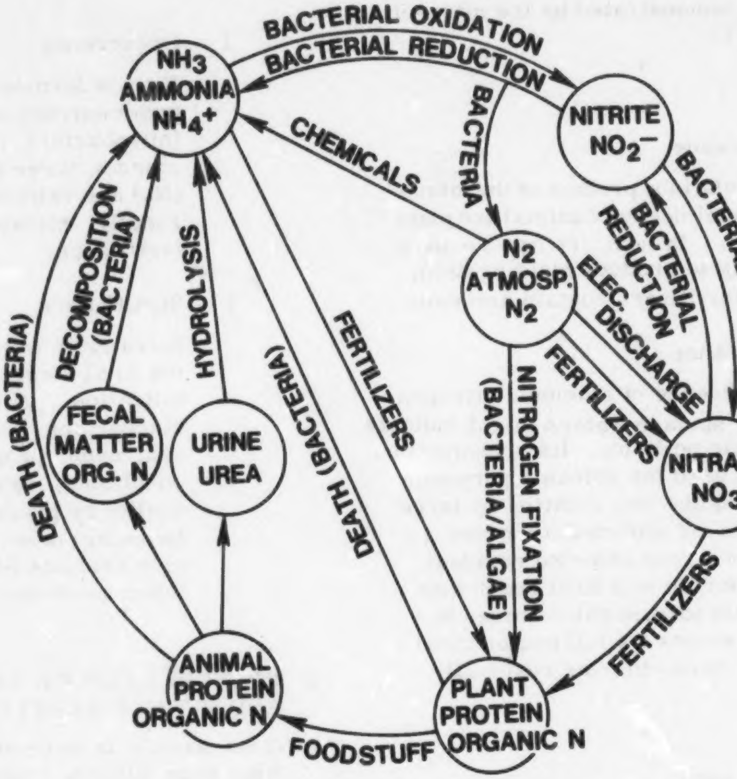
B For Ammonia and Nitrate, the storage time can be extended by lowering the sample pH to less than 2 by the addition of concentrated sulfuric acid and storing at 4°C. (2 ml of acid per liter is usually sufficient. Check with pH paper).

C Mercuric chloride is effective as a preservative, but its use is discouraged because:

1 The Hg ion interferes with some of the nitrogen tests.

2 The Hg presents a disposal problem.

D Even when "preserved", conversion from one nitrogen form to another may occur. Samples should be analyzed as soon as possible.


BACTERIAL OXIDATION
BACTERIAL REDUCTION
NH3 AMMONIA NH4+
NITRITE NO2−
CHEMICALS
BACTERIA
BACTERIAL REDUCTION
BACTERIAL OXIDATION
N2 ATMOSP. N2
ELEC. DISCHARGE
DECOMPOSITION (BACTERIA)
HYDROLYSIS
DEATH (BACTERIA)
FERTILIZERS
FERTILIZERS
FECAL MATTER ORG. N
URINE UREA
NITRATE NO3−
NITROGEN FIXATION (BACTERIA/ALGAE)
DEATH (BACTERIA)
FERTILIZERS
ANIMAL PROTEIN ORGANIC N
PLANT PROTEIN ORGANIC N
FOODSTUFF

THE NITROGEN CYCLE

Figure 1

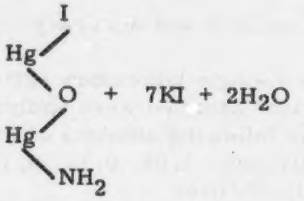
III DETERMINATION OF AMMONIA

A Nesslerization

1 Reaction

Nessler's reagent, a strong alkaline solution of potassium mercuric iodide, combines with NH_3 in alkaline solution to form a yellowish brown colloidal dispersion.

$$2K_2HgI_4 + NH_3 + 3KOH \longrightarrow$$



Yellow-Brown

The intensity of the color follows the Beer-Lambert Law and exhibits maximum absorption at 425 nm.

2 Interferences

a Nessler's reagent forms a precipitate with some ions (e.g., Ca^{++}, Mg^{++}, Fe^{+++}, and $S^{=}$). These ions can be eliminated in a pretreatment flocculation step with zinc sulfate and alkali. Also, EDTA or Rochelle salt solution prevents precipitation with Ca^{++} or Mg^{++}.

b Residual chlorine indicates ammonia may be present in the form of chloramines. The addition of sodium thiosulfate will convert these chloramines to ammonia.

c Certain organics may produce an off color with Nessler's reagent. If these compounds are not steam distillable, the interference may be eliminated in the distillation method.

d Many organic and inorganic compounds cause a turbidity interference in this colorimetric test. Therefore direct nesslerization is not a recognized method. The following distillation procedure is a required, preliminary treatment.

B Distillation (6, 7, 8)

1 Reaction

a The sample is distilled in the presence of a borate buffer at pH 9.5

$$NH_4 + \xrightarrow{\Delta} NH_3 + H^+$$

$$H^+ + Na_2B_4O_7 \longrightarrow \text{pH 9.5 maintained}$$

Buffer

b The ammonia in the distillate is then measured by either of two techniques.

1) Nesslerization is used for samples containing less than 1 mg/l of ammonia nitrogen.(6, 7, 8)

2) Absorption of NH_3 by boric acid and back titration with a standard strong acid is more suitable for samples containing more than 1 mg $NH_3{-}N$/l(6, 7, 8)

$$NH_3 + HBO_2 \longrightarrow NH_4^+ + BO_2^-$$

$$BO_2^- + H^+ \xrightarrow[+]{\text{Methyl Red}} HBO_2^-$$

Methylene Blue

pH 7.8 - 8.0	pH 6.8 - 7.0
Green	Purple

2 Interferences

a Cynate may hydrolyze, even at pH 9.5.

b Volatile organics may come over in the distillate, causing an off-color for Nesslerization. Aliphatic and aromatic amines cause positive interference by reacting in the acid titration. Some of these can be boiled off at pH 2 to 3 prior to distillation.

c Residual chlorine must be removed by pretreatment with sodium thiosulfate.

d If a mercury salt was used for preservation, the mercury ion must be complexed with sodium thiosulfate (0.2 g) prior to distillation.

3 Precision and Accuracy(8)

Twenty-four analysts in sixteen laboratories analyzed natural water samples containing the following amounts of ammonia nitrogen: 0.21, 0.26, 1.71, and 1.92 mg NH_3-N/liter.

a Precision

The standard deviation was: 0.122, 0.070, 0.244, and 0.279 mg NH_3-N/liter, respectively.

b Accuracy

The bias was: -0.01, -0.05, +0.01, and -0.04 mg NH_3-N/liter, respectively.

C Selective Ion Electrode(8)

1 Principle

A hydrophobic, gas-permeable membrane is used to separate the sample solution from an ammonium chloride internal solution. The ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.

2 Interferences

a Volatile amines are a positive interference.

b Mercury forms a complex with ammonia so it should not be used as a preservative.

3 Precision and Accuracy

In a single laboratory (EPA) four surface water samples were analyzed containing the following amounts of ammonia nitrogen: 1.00, 0.77, 0.19, and 0.13 mg NH_3-N/liter.

a Precision

The standard deviations were 0.038, 0.017, 0.007, and 0.003 mg NH_3-N/liter, respectively.

b Accuracy

The % recovery on the 0.19 and 0.13 concentrations was 96% and 91% respectively.

D NPDES Ammonia Methodology

Manual distillation is not required if comparability data on representative effluent samples are on company file to show that this preliminary distillation is not necessary. However, manual distillation will be required to resolve any controversies.

Nesslerization, titration, and the selective ion electrode are all recognized methods. The automated phenolate method is also cited.

IV DETERMINATION OF NITRITE

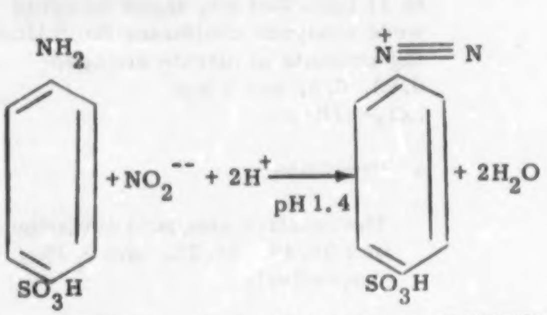
A Diazotization(6, 8)

1 Reaction

a Under acid conditions, nitrite ions react with sulfanilic acid to form a diazo compound.

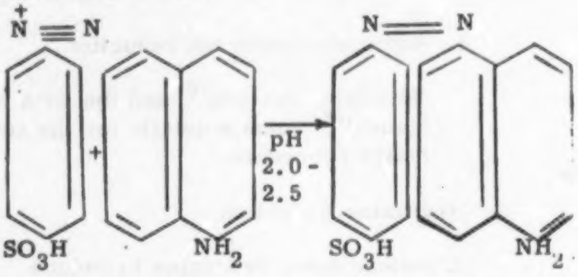
b The diazo compound then couples with α-naphthylamine to form an intense red azo dye which exhibits maximum absorption at 540 nm.

c Diazotization of Sulfanilamide



SULFANILIC ACID DIAZONIUM COMPOUND

d Coupling



intense red azo dye

2 Interferences

a There are very few known interferences at concentrations less than 1000 times that of nitrite.

b High alkalinity (greater than 600 mg/liter) will give low results due to a shift in pH.

c Strong oxidants or reductants in the sample also give low results.

3 Precision

On a synthetic sample containing 0.25 mg nitrite nitrogen/1, the ARS Water Minerals Study (1961) reported 125 results with a standard deviation of ± 0.029 mg/1.

B NPDES Nitrite Methodology

The colorimetric diazotization method, either manual or automated, is the only one cited in the Federal Register.

V DETERMINATION OF NITRATE

A Brucine Sulfate(6, 7, 8)

1 Reaction

Brucine sulfate reacts with nitrate in a 13N sulfuric acid solution to form a yellow complex which exhibits maximum absorption at 410 nm. Temperature control of the color reaction is extremely critical. The reaction does not always follow Beer's Law. However, a modification by Jenkins and Medsker(2) has been developed. Conditions are controlled in the reaction so that Beer's Law is followed for concentrations from 0.1 to 2 mg nitrate N/liter. The ideal range is from 0.1 to 1 mg NO_3-N/liter.

2 Interferences

a Nitrite may react the same as nitrate but can be eliminated by the addition of sulfanilic acid to the brucine reagent.

b Organic nitrogen compounds may hydrolyze and give positive interference at low (less than 1 mg/1) nitrate nitrogen concentrations. A correction factor can be determined by running a duplicate of the sample with all the reagents except the brucine-sulfanilic acid.

c Residual chlorine may be eliminated by the addition of sodium arsenite.

d Strong oxidizing or reducing agents interfere.

e The effect of salinity is eliminated by addition of sodium chloride to the blanks, standards, and samples.

3 Precision and Accuracy(8)

a Twenty-seven analysts in 15 laboratories analyzed natural water samples containing the following increments of inorganic nitrate: 0.16, 0.19, 1.08 and 1.24 mg N/liter.

b Precision results as standard deviation were 0.092, 0.083, 0.245, and 0.214 mg N/liter respectively.

c Accuracy expressed as bias was -0.01, +0.02, +0.04 and +0.04 mg N/liter, respectively.

B Cadmium Reduction(6, 8)

1 Reaction

A non-turbid sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form an intensely colored azo dye which is measured spectrophotometrically.

To obtain the value for only nitrate, more of the non-turbid sample is tested using the same colorimetric reaction but without passing it through the reduction column. The resulting value represents the nitrite originally present in the sample. Subtracting this nitrite value for the non-reduced sample from the nitrate + nitrite value for the reduced sample gives the value for nitrate originally present in the sample.

2 Interferences

a Build-up of suspended matter in the reduction column will restrict sample flow. Filtration or flocculation with zinc sulfate should remove turbidity.

b High concentrations of iron, copper or other metals may interfere. EDTA is used to complex these.

c Large concentrations of oil and grease in a sample can coat the surface of the cadmium. Pre-extracting the sample with an organic solvent removes oil and grease.

3 Precision and accuracy(6)

In 11 laboratories, three samples were analyzed containing the following amounts of nitrate nitrogen: 0.05, 0.5, and 5 mg NO_3-N/liter.

a Precision

The relative standard deviation was 96.4%, 25.6%, and 9.2%, respectively.

b Accuracy

The relative error was 47.3%, 6.4%, and 1.0%, respectively.

4 Automated cadmium reduction

Standard Methods(6) and the EPA Methods Manual(8) contain details for the automated procedure.

C Hydrazine Reduction

A method using hydrazine to reduce nitrate to nitrite followed by subsequent measurement of nitrite by diazotization was reported by Fishman, et al.(1)

The means to determine nitrate is the same as above in the Cadmium Reduction Method. Subtraction of nitrite (determined from non-reduced sample) from the total nitrite (reduced nitrate + original nitrite) will give the original nitrate nitrogen concentration.

The procedure was adapted to the Auto Analyzer by Kamphake, et al.(3) It is available from Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, Ohio, 45268.

D Compliance Nitrate Methodology

1 NPDES/Certifications

The Federal Register lists the brucine sulfate, cadmium reduction and automated cadmium reduction or hydrazine reduction methods.

2 Drinking Water

The National Interim Primary Drinking Water Regulations list one brucine sulfate method (13th ed. of reference 6) and one codmium reduction method (reference 8). Amendments to the NIP DWR, August 27, 1980, p. 57344 added the automated hydrazine reduction method (1971 ed. of reference 8).

REFERENCES

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2 Jenkins, David and Medsker, Lloyd L. Brucine Method for Determination of Nitrate in Ocean, Estuarine and Fresh Waters. Anal. Chem. 36:610-612. March, 1964.

3 Kamphake, L. J., Hannah, S. and Cohen, J. Automated Analysis for Nitrate by Hydrazine Reduction. Water Research, 1, 205. 1967.

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6 Standard Methods for the Examination of Water and Waste Water. APHA. AWWA. WPCF. 14th Ed, 1976.

7 Annual Book of Standards, Part 31, Water, 1975.

8 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environment Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by B. A. Punghorst, former Chemist, and C. R. Feldmann, Chemist, and updated by A. D. Kroner, Chemist, National Training and Operational Technology Center, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Ammonia, Chemical Analysis, Nitrates, Nitrites, Nitrogen, Nitrogen Compounds, Nitrogen Cycle, Nutrients, Water Analysis, Water Pollution Sources

DETERMINATION OF KJELDAHL NITROGEN
(MICRO APPARATUS-NESSLERIZATION)

I REAGENTS

A Distilled Water

This should be ammonia-free. Pass distilled water through an ion exchange column with strongly acidic cation resin mixed with a strongly basic anion resin.

B Sulfuric Acid (20%)

20 ml acid/ 100 ml distilled water

C Mercuric Sulfate Solution

Dissolve 8g mercuric oxide in 50 ml of 20% sulfuric acid. Dilute to 100 ml with distilled water.

D Digestion Reagent

Dissolve 134g potassium sulfate in about 650 ml distilled water. Add 200 ml concentrated sulfuric acid. Add 25 ml of mercuric sulfate solution (C above) and dilute to 1 liter.

E Sodium Hydroxide - Sodium Thiosulfate Solution

Dissolve 500g sodium hydroxide and 25g sodium thiosulfate pentahydrate in distilled water and dilute to 1 liter.

F Boric Acid Solution, 2%

G Ammonium Chloride Stock Solution

Dissolve 3.819g NH_4Cl in distilled water and dilute to 1 liter. 1.0 ml = 1.0 mg NH_3-N.

H Ammonium Chloride Standard Solution

Dilute 10.0 ml of the stock solution with distilled water to 1 liter.
1.0 ml = 0.01 mg NH_3-N

I Sodium Hydroxide Solution

Dissolve 160 g sodium hydroxide in 500 ml distilled water.

J Nessler Reagent

Dissolve 100g mercuric iodide and 70g potassium iodide in a small volume of water. Add this mixture slowly to the sodium hydroxide solution (J above), then dilute to 1 liter.

II EQUIPMENT PREPARATION

A This procedure should be used if the apparatus has been out of service for 4 hours or more.

1 Add about 50 ml of a 1:1 mixture of ammonia-free distilled water and sodium hydroxide-sodium thiosulfate solution to each of the micro Kjeldahl flasks to be used.

2 Add glass beads to each flask.

3 Attach a flask to the steam distillation apparatus and distill about half the mixture.

4 Add 1 ml of the Nessler reagent to the distillate to check for ammonia.

   a If the distillate is colorless, the equipment is ammonia-free and the procedure can be repeated with the next flask to be used.

   b If the distillate is yellow, discard it, distill another half of the mixture and check this distillate with 1 ml Nessler reagent. Repeat the process until the distillate is colorless.

III DIGESTION OF SAMPLE

A Preparation of Digestion Mixture

1 Shake the sample.

2 Measure 50.0 ml sample into a 100 ml Kjeldahl flask.

3 Add 10 glass beads.

4 Add 10 ml of the digestion reagent.

B Digestion

1 Place the flask in a properly ventilated Kjeldahl digestion apparatus.

2 Turn on the heat source.

3 Evaporate the mixture until sulfur trioxide (SO_3) fumes are given off. (SO_3) fumes are white and TOXIC. Also, the solution will be pale yellow.)

4 Continue heating for an additional 30 minutes.

5 Turn off the heat source.

6 Cool the residue in the flask.

IV STEAM DISTILLATION

A Preparation of the Digestion Residue

1 Add 30 ml of ammonia-free distilled water to the digested residue in the Kjeldahl flask.

2 Connect the flask to the ground glass joint of the micro steam distillation apparatus.

3 Measure 35 ml of water in a graduate, pour it into a 50 ml Nessler tube and mark the tube at the 35 ml meniscus. Empty the tube.

4 Add 5 ml of 2% boric acid to the 50 ml Nessler tube.

5 Position the Nessler tube so that the tip of the condesnser is below the level of the boric acid solution in the tube.

6 Carefully add 10 ml of the sodium hydroxide-sodium thiosulfate solution from the dropping funnel.

B Distillation

1 Turn on the heat source.

2 Distill at a rate of 6-10 ml/minute up to the 35 ml mark on the Nessler tube.

3 Remove the receiving flask.

4 Put a small beaker under the condenser tip to receive any additional distillate.

5 Turn off the heat source if there are no more digestion residues to distill.

V COLORIMETRY FOR AMMONIA-NESSLERIZATION

If the ammonia content is found to be greater than 1 mg/liter, a titration procedure should be used(1) rather than Nesslerization.

A Preparation of Standards and Sample

1 Label five 50.0 ml Nessler tubes with the following: 0, 2, 5, 8, and 10.

2 Pipet the following volumes of ammonium chloride standard solution into the correspondingly labeled tubes: 2.0 ml, 5.0 ml, 8.0 ml, and 10.0 ml.

3 Mark "S" on the Nessler tube containing 35 ml of distillate.

4 Pour ammonia-free distilled water into the tube labeled "0", bringing the volume to the 50.0 ml line.

5 Add ammonia-free distilled water to each of the remaining tubes, bringing the volume of each to the 50.0 ml line.

6 Add 1.0 ml of Nessler Reagent to each of the 9 tubes.

7 Mix the solution in the "0" tube using a cap or a rubber stopper on the top, then inverting the tube three times.

8 Remove, rinse, and dry the cap or stopper.

9 Repeat steps 7 and 8 to mix the contents of the other 8 tubes.

10 After mixing, let the tubes stand in a rack for 20 minutes before getting absorbance readings.

B Spectrophotometric Readings

1 Turn the instrument on.

2 Set the wavelength at 425 nm.

3 After the 20 minute time span, use the contents of the "0" tube to adjust to zero absorbance on the spectrophotometer.

4 Using the contents of the tube labeled 0.5, rinse, then fill an instrument cell.

5 Place the cell in the holder and record the absorbance value from the instrument.

6 Discard the contents from the cell.

7 Use ammonia-free distilled water to rinse the cell three times.

8 Repeat steps 4 through 7 to obtain absorbance values for the rest of the standards and for the sample.

9 Turn off the instrument.

VI CALIBRATION CURVE

A Constructing the Curve

1 Calculate the concentration of each standard by multiplying the ml of working standard used times 0.01 mg/ml, which is the concentration of the standard solution. This was diluted to 50.0 ml in the Nessler tube, so the result is mg NH_3-N/50.0 ml. For example, if 0.5 ml of standard was used, the concentration is (0.5)(0.01) = 0.005 mg NH_3-N/50.0 ml.

2 Plot the absorbance values for the standards against these calculated concentrations.

3 Draw the best straight line from zero through all the points.

B Using the Curve

1 To find the NH_3-N concentration in the sample, locate its absorbance value on the curve.

2 Find the corresponding mg NH_3-N/50.0 ml by dropping a vertical line to the concentration axis.

3 Record this result.

VII FINAL CALCULATIONS

A Use this formula to calculate Total Kjeldahl Nitrogen:

$$\text{TKN, mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

Where:

A = mg NH_3-N/50.0 ml from curve
B = ml total distillate including boric acid
C = ml distillate taken for Nesslerization
ml sample = ml of original sample taken

B An example calculation using the value from the calibration curve would be:

$$\text{TKN, mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

If:

A = 0.045
B = 35 ml (30 ml distillate + 5 ml boric acid)
C = 35 ml
ml sample = 50 ml

Then:

$$\text{TKN, mg/l} = \frac{0.045 \times \overset{20}{\cancel{1000}}}{\underset{1}{\cancel{50}}} \times \frac{\overset{1}{\cancel{55}}}{\underset{1}{\cancel{55}}}$$

$$= 0.045 \times 20 \times 1$$
$$= 0.045 \times 20$$
$$= 0.90$$

TKN = 0.90 mg/l

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environment Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Ammonia, Analytical Techniques, Chemical Analysis, Laboratory Tests, Nitrogen Nitrogen Compounds, Nutrients, Water Analysis

DATA SHEET

ml of ammonium Chloride standard solution used	mg of NH_3 - N/50 ml	Absorbance
0.0		0.
0.5		0.
1.0		0.
2.0		0.
4.0		0.
5.0		0.
8.0		0.
10.0		0.

Absorbance of sample = 0.__________

A = mg NH_3 - N/50 ml from curve = __________

B = ml total distillate including boric acid = __________

C = ml distillate taken for nesslerization = __________

ml sample = ml of original sample taken = __________

$$\text{TKN, in mg/liter} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

$$= \frac{\quad \times 1000}{\qquad\qquad} \times \underline{\qquad\qquad}$$

= __________

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.

2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This ratio varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Sample	5-Day BOD-mg/mg	COD-mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	.786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	.14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1.97	69
Phenol - C_6H_6O	.05 to 2.1 depending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1.15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1.60	61
Secondary Effluent, Clarified	13*	75*	21*
" "	23*	67*	12*
" " "	4*	36*	7*

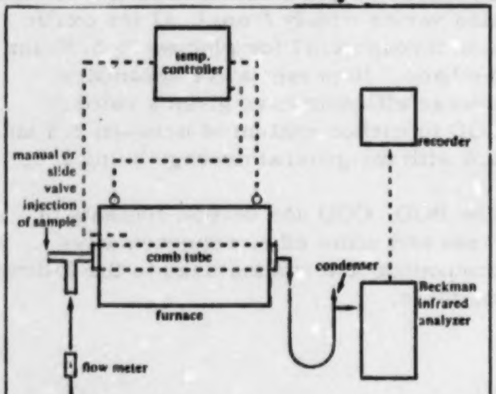
*In units of mg/l

III THE INFRA-RED TYPE CARBON ANALYZER

A Principle of Operation

Basically the infra-red carbonaceous analyzer made by Beckman, consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer and a strip-chart recorder.

Infra-red Carbonaceous Analyzer Schematic


temp. controller
recorder
manual or slide valve injection of sample
comb tube
condenser
Beckman infrared analyzer
furnace
flow meter
oxygen carrier from cylinder

A micro sample (20-40 μl) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen or CO_2-free air. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2, oxygen or air, and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2. The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter.

B Application

Results (1) show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by determination of carbon both before and after the sample solution has been blown with a carbon-free gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation.

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HCl per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2. Five minutes of blowing with a gas free of carbon sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference.

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a nonacidified sample into the high temperature furnace yields a peak representing the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor, resulting from vaporization of the sample, causes a slight interference in the method. Most of the water is trapped out by the air cooled condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation. In tests of solutions containing the following anions: NO_3^-, Cl^-, SO^{-2}, PO_4^{-3}, no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent. The minimum detectable concentration using the prescribed operating instructions is 1 mg/1 carbon. Generally, the data are reproducible to ± 1 mg/1 with a standard deviation of 0.7 mg/1 at the 100 mg/1 level.

V THE FLAME IONIZATION TYPE CARBON ANALYZER

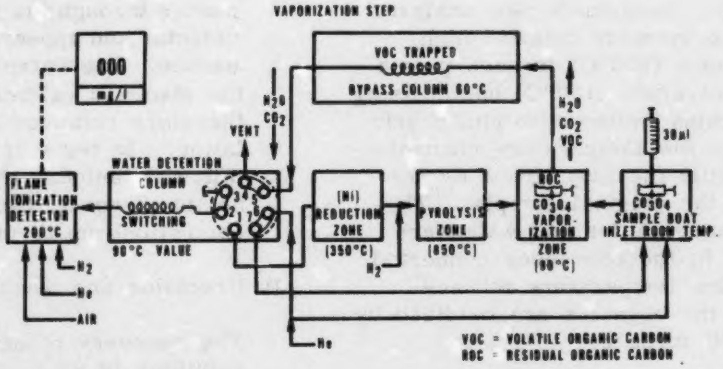
An example of a flame ionization carbonaceous analyzer is the one produced by Dohrmann.

To determine TOTAL ORGANIC CARBON a 30 μl acidified water sample is injected into a sample boat containing a cobalt oxide oxidizer at room temperature. The boat is then advanced to the 90° C vaporization zone where H_2O, CO_2 (from dissolved CO_2, carbonates and bicarbonates) and organic carbon materials which are volatile at 90° C are swept into the bypass column. Here volatile organic carbon (VOC) is trapped on a Porapak Q column at 60° C while the H_2O and CO_2 are swept through the switching valve and vented to atmosphere.

After sample vaporization, the valve is automatically switched to the pyrolyze position and the boat is then advanced to the pyolysis zone. Residual organic carbon (ROC) materials left in the boat react with the Co_3O_4 at 850° C to produce CO_2. At the same time the bypass column is backflushed at 120°C thus sweeping the VOC material through the pyrolysis zone. Both the VOC and the CO_2 (from the ROC) are swept by helium into the hydrogen enriched nickel catalyst reduction zone where all carbon is converted to methane at 350° C.

The reduction product is swept through the switching valve, the water detention column and into the flame ionization detector which responds linearly to methane. The detector output is integrated and displayed in milligrams per liter (mg/l) or parts per million (ppm) on the digital meter.

To determine total carbon, simply set the function switch to TOTAL CARBON, and cycle an unacidified sample through the vaporize and pyrolyze steps. The switching valve remains in the pyrolyze position, directing ALL carbonaceous matter to the detector.


VAPORIZATION STEP
000
mg/l
VOC TRAPPED
BYPASS COLUMN 60°C
H_2O
CO_2
VENT
H_2O
CO_2
VOC
30μl
FLAME IONIZATION DETECTOR 200°C
WATER DETENTION COLUMN
SWITCHING
90°C VALUE
1 2 3 4 5 6 7
(Ni) REDUCTION ZONE (350°C)
PYROLYSIS ZONE (850°C)
ROC
Co_3O_4
VAPORIZATION ZONE (90°C)
Co_3O_4
SAMPLE BOAT INLET ROOM TEMP.
H_2
He
AIR
H_2
He
VOC = VOLATILE ORGANIC CARBON
ROC = RESIDUAL ORGANIC CARBON

VI THE AUTOCLAVE TYPE OF CARBON ANALYZER

Oceanography International makes a TOC apparatus in which the sample is placed in an ampule that contains phosphoric acid and potassuum persulfate. The ampule is flame sealed and autoclaved. The tip of the ampule is then broken and the CO_2 removed by a gas stream that carries the CO_2 from the sample to an infrared detector. The autoclave digestion approximates a COD in which the COD's H_2SO_4 is replaced with H_3PO_4 and $K_2Cr_2O_7$ is replaced by $K_2S_2O_8$. This instrument does not have difficulty with salts coating a catalyst, but has a comparatively high time requirement.

VII LOW ORGANIC CARBON LEVELS

The organic carbon in solution can be measured down to levels as low as 50 ppb using an instrument manufactured by Barnstead. Samples up to 100 ml in volume are introduced into the instrument. The sample is introduced into the system with a syringe or pipet. Inorganic carbon in the form of dissolved CO_2 is first stripped out of the sample by a stream of air (acidification is used to bring the pH to 4.2). The air carries the inorganic carbon (CO_2) to a measuring chamber containing 18 megohm-cm, zero-organic water. The CO_2 dissolves in the pure water and the resultant change in specific resistance is measured and stored in memory. See the figure on page 17-6.

Following the determination of inorganic carbon, the ultra-violet lamp within the irradiation chamber is energized. All dissolved organic compunds - both volatile and non-volatile - are completely oxidized. The resulting CO_2 is carried by the air stream to the measuring chamber where it also dissolves in the water. The unit again measures the resistivity of the water within the measuring chamber to determine the total carbon of the sample. The system now automatically subtracts the stored inorganic carbon value from the total carbon value and displays the difference, which is the level of organic carbon in the sample.

After the result has been displayed, the unit automatically begins a clean-up cycle in which all CO_2 and other contaminants in the system are removed by an ion-exchange cartridge. Once all water within the system has returned to an 18 megohm-cm, zero-organic condition, the system is ready for the next sample.

Dohrmann has a ultra-violet lamp system for low organic carbon levels that connects along side of their regular analyzer. The CO_2 from the ultra-violet lamp component passes to the regular analyzer and its flame ionization detector.

Ultra-violet systems will give low results if the organic carbon is not in solution.

VIII APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below:

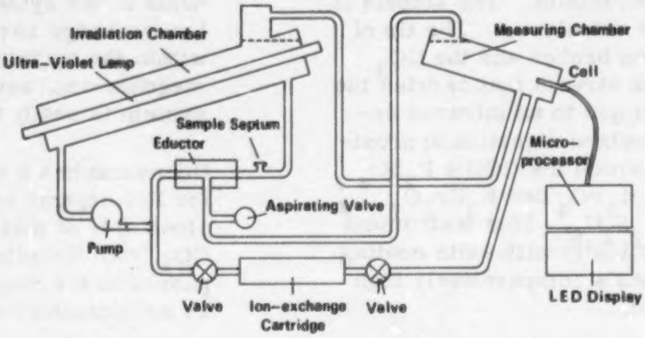
A Determine the efficiency of various waste-water renovation processes, both in the laboratory and in the field.

B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed.

C Monitoring a waste stream to check for product loss.

D Follow the rate of utilization of organic nutrients by micro-organisms.

E To detect organic impurities in inorganic compounds.


Irradiation Chamber
Ultra-Violet Lamp
Sample Septum
Eductor
Aspirating Valve
Pump
Valve
Ion-exchange Cartridge
Valve
Measuring Chamber
Cell
Micro-processor
LED Display

Ultra-violet low organic carbon Barnstead apparatus.

A summary of some TOC laboratory instrument:-

Instrument Manufacturer	Mode of Oxidation	Detector
Beckman	950°C furnace and catalyst of Co oxides	Infra-red
Oceanography International	H_3PO_4-$K_2S_2O_8$ plus autoclave digestion	Infra-red
Astro, sold by Curtin Matheson	850°C combustion chamber	Infra-red
Dohrmann-Envirotech	850° furnace plus reduction to methane	Flame ionization
	(Low Organic Carbon Levels)	
Dohrmann-Envirotech	Ultra-violet lamp, H_3PO_4	Returned to unit with flame ionization
Barnstead	Ultra-violet lamp, H_3PO_4	Conductivity

Note that there may be other manufacturers of TOC equipment.

IX ADVANTAGES OF CARBON ANLYZER

A Speed

The Carbonaceous Analyzer's most important advantage is its speed of analysis. One analysis can be performed in 2 - 3 minutes for a channel on the Beckman instrument or double that on the Dohrman. This speed of analysis brings about economy of operation. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon. No compound has been found to which the method is inapplicable.

X CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

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5 Various manufacturers' literature.

This outline was prepared by Robert T. Williams, Chief, and revised by Charles J. Moench, Jr., Waste Identification and Analysis Section, MERL, USEPA, Cincinnati, Ohio 45268.

Descriptors: Biochemical Oxygen Demand, Carbon, Chemical Analysis, Chemical Oxygen Demand, Organic Matter, Organic Wastes, Water Analysis, Instrumentation, Nutrients

CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

A The Chemical Oxygen Demand (COD) test is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation under specific conditions of oxidizing agent, temperature and time.

B A variety of terms have been and are used for the test described here as COD:

1 Oxygen absorbed (OA) primarily in British practice.

2 Oxygen consumed (OC) preferred by some, but unpopular.

3 Chemical oxygen demand (COD) current preference.

4 Complete oxygen demand (COD) misnomer.

5 Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.

6 Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.

C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.

1 Ceric sulfate has been investigated, but in general it is not a strong oxidant.

2 Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.

a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.

3 Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.

4 A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure.(1, 2) Statistical comparisons with other methods are described. (3)

5 Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.

a Van Slyke(4) described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.

b Van Hall, et al., (5) used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.

6 Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe^{+2}, SH.
Van Slyke Carbon detn.	400+	1 hr.	H_3PO_4 HIO_3 H_2SO_4 $K_2Cr_2O_7$ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH_3 oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

1 Any relationships among COD & BOD or any other tests are fortuitous because the tests measure the oxidizability of a given sample under specified conditions, which are different for each test.

2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.

b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.

c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.

d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST(2) AS RELATED TO BOD

A Advantages

1 Time, manipulation, and equipment costs are lower for the COD test.

2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3 COD test conditions can be standardized more readily to give more precise results.

4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.

5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.

6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters.

B Limitations

1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.

2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.

5 It is not advisable to expect precise COD results on saline water.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

A The COD procedure (1) considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers(6) suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg. Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be 300 -(200 x 0.23)or 300 - 46 = 254 mg COD/1 on a chloride corrected basis.

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4, and continue the reflux or use of $HgSO_4$(D).

D Dobbs and Williams (7) proposed prior complexation of chlorides with $HgSO_4$ to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the $HgSO_4$ complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations.

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides. Sea water contains 18000 to 21000 mgCl^-/1 normally. Equivalent chloride correction for COD exceeds 4000 mg/l. The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with $HgSO_4$ may give equally confusing results.

$HgSO_4$ appears to give precise results for COD when chlorides do not exceed about 2000 mg/l. Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test. This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical. Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 15 mg/liter and a chloride concentration less than 2000 mg/liter), the EPA specifies the procedures found in Standard Methods (2) and in ASTM(8).

2 For low level COD (samples with less than 15 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure (9). The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 2000 mg/liter), EPA provides an analytical procedure(9) involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination.

V PRECISION AND ACCURACY(9)

Eighty six analysts in 58 laboratories analyzed a distilled water solution containing oxidizable organic material equivalent to 270 mg/l COD. The standard deviation was ± 17.76 mg/l COD with an accuracy as percent relative error (bias) of −4.7%. For a solution equivalent to 12.3 mg/l COD (low level), the standard deviation was ± 4.15 mg/l with an accuracy as percent relative error (bias) of 0.3%. (EPA Method Research Study 3)

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE:

A Sample size and COD limits for 0.250 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagent for COD's in the range of 5-50 mg/1, (low level).

Sample Size	mg COD/1
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid but the orange color of dichromate should remain. If the sample color changes from orange to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples so appropriate analytical techniques can be used.

D Special precautions advisable for the regular COD procdure and essential when using 0.025 N reagents include:

1 Keep the apparatus assembled when not in use.

2 Plug the condenser breather tube with glass wool to minimze dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components.

5 Distilled water and sulfuric acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant.

VII NPDES METHODOLOGY

Under the National Pollutant Discharge Elimination System, the accepted method (Federal Register, vol. 41, no. 232, part II, Wednesday, Dec. 1, 1976) for doing the chemical oxygen demand test is given in Standard Methods (2), p. 550; ASTM (8), p. 472; & the EPA manual (9), p. 20.

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from prior outlines by R. C. Kroner, R. J. Lishka, and J. W. Mandia.

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This outline was prepared by F.J. Ludzack, Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analysis, Biochemical Oxygen Demand, Chemical Analysis, Chemical Oxygen Demand, Chlorides, Oxygen Demand, Wastewater, Water Analysis

LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A **Standard Potassium Dichromate (0.250 N):**

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which has been dried at 103° C for two hours, in distilled water and dilute to one liter.

B **Ferrous Ammonium Sulfate (0.1N):**

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water.
Carefully add 20 ml of concentrated H_2SO_4. Cool and dilute to one liter.

C **Ferroin Indicator:**

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D **Concentrated Sulfuric Acid (36 N):**

E **Mercuric Sulfate:** Analytical Grade

F **Silver Sulfate:** Analytical Grade

G **Concentrated Sulfuric Acid - Silver Sulfate:**

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (4-5 hours, with stirring, for dissolution)

II EQUIPMENT PREPARATION(1)

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (300 mm jacket Liebig, West or equivalent) with 24/40 standard taper joint, should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several boiling stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, re-assemble the apparatus. The top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION(2) OF FERROUS AMMONIUM SULFATE

A Measure 10.0 ml of the standard potassium dichromate sol. into a 500 ml, wide mouth Erlenmeyer flask.

B Add about 90 ml distilled water and mix.

C Add 30 ml of concentrated H_2SO_4 and cool the mixture to room temperature.

D Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate sol..

E Calculate the normality, N, of the ferrous ammonium sulfate sol.:

$$\text{N of } Fe(NH_4)_2(SO_4)_2 = \frac{\text{ml } K_2Cr_2O_7 \times \text{N of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE

A Pipet 20 ml of sample (or pipet a smaller portion of sample and add enough distilled water to make a 20 ml volume) into a 500 ml 24/40 standard taper Erlenmeyer flask.

B Add 0.4 g mercuric sulfate.

C Add 6-10 boiling chips.

D Slowly add 3 ml of concentrated sulfuric acid and swirl to dissolve the mercuric sulfate. (Some solid may remain). Cool while mixing.

E Add 10.0 ml of the 0.250 N potassium dichromate sol. and mix.

F Attach the flask to the condenser and start the cooling water.

G Slowly add 27 ml of the sulfuric acid-silver sulfate reagent through the open end of the condenser. Try to mix the contents while adding the acid.

H CAUTION: After adding the acid, swirl the flask to thoroughly mix the contents. Otherwise,local heating can occur in the bottom of the flask and the mixture may be blown out of the condenser.

I Plug the open end of the condenser with glass wool or cover with a small beaker (depending on construction) to prevent intrusion of contaminants.

J Turn on the heat source and reflux the mixture for 2 hours after boiling begins.

K Turn off the heat and allow the solution to cool. Then turn off cooling water.

L Wash down the inside walls of the condenser with distilled water.

M With a twisting motion, disconnect the condenser from the flask, then rinse the joint so the rinses go into the flask.

N Add distilled water to the flask for a total volume of about 130 ml.

O If the mixture is still warm, cool to room temperature.

P Add 2-3 drops of ferroin indicator and mix.

Q Titrate the excess dichromate with standard ferrous ammonium sulfate sol. to a reddish brown end point.

R A blank consisting of 20 ml of distilled water and containing all the test reagents is to be refluxed and titrated in the same manner as the sample.

V CALCULATION

$$\text{mg COD/l} = \frac{(A-B)\,N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml Fe $(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from outlines prepared by R. J. Lishka and Charles R. Feldmann.

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This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Chemical Oxygen Demand, Organic Compounds, Oxidation, Oxygen, Oxygen Demand, Oxygen Requirements, Water Analysis

DATA SHEET

ml of $K_2Cr_2O_7$ used to standardize the ferrous ammonium sulfate (FAS) = 10.0

N of $K_2Cr_2O_7$ = 0.25

ml of FAS used to titrate the $K_2Cr_2O_7$ = ________

$$\text{N FAS} = \frac{10.0 \times 0.25}{\text{ml FAS}}$$

$$= \frac{2.5}{}$$

= ________

ml FAS used for blank, a = ________

ml FAS used for sample, b = ________

ml sample = ________

$$\text{COD, in mg liter} = \frac{(a - b) \times \text{N FAS} \times 8 \times 1000}{\text{ml sample}}$$

$$= \frac{(\quad - \quad) \times \quad \times 8 \times 1000}{}$$

= ________

DETERMINATION OF SURFACTANTS

I NATURE OF SURFACTANTS AND SYNTHETIC DETERGENTS

A Definitions

1 Detergents

The dictionary states that the verb, deterge, means to wash off or to cleanse. Detergency denotes a cleansing power or quality.

Larson (5) characterizes a good detergent as a material which is water soluble, permits the water solution to penetrate capillaries by lowering interfacial tension (wetting action), breaks up or separates particles having agglomerated (dispersing action), and links the dirt or oil particles with the water (emulsifying action).

2 Surface active material (Surfactant)

This term is reserved for those organic compounds which exhibit detergent properties plus stability toward hardness. They are able to alter the surface or interfacial properties of their solutions to an unusual extent, even when present in low concentrations.

3 Synthetic detergents

The term, synthetic detergent, is not rigorously defined. In general, however, it is a material which contains a surfactant plus one or more builders.

4 Builders

A builder is a compound which is used to enhance the cleansing characteristics of a synthetic detergent.

Sodium sulfate, sodium silicate, sodium chloride, sodium tripolyphosphate, and carboxymethyl cellulose are examples of builders which might be found in household or industrial synthetic detergents.

B Chemical Behavior of Surfactants

On the basis of their ionization in water, surfactants may be classified as anionic, cationic and nonionic.

1 Anionic surfactants

These are the most widely used in the manufacture of synthetic detergents and are therefore the greatest contributors to pollution. They are characterized by the fact that they ionize in water to give an anion of large size and mass and a cation of small size and mass.

a Soap exhibits detergent properties and ionizes as described above, but has become less popular for detergent use because of its lack of stability toward hardness.

$$C_{11}H_{23}CO_2Na \rightarrow C_{11}H_{23}CO_2^- + Na^+$$

(Soap) (Anion) (Cation)

b The alkyl aryl sulfonates represent a second type of anionic surfactant.

$$R\text{-}C_6H_4\text{-}SO_3Na \rightarrow R\text{-}C_6H_4\text{-}SO_3^- + Na^+$$

(Alkyl aryl sulfonate) (Anion) (Cation)

The C_6H_4 grouping represents a benzene ring; the R represents a chain of carbon atoms. If the chain is "straight" the alkyl aryl sulfonate is referred to as LAS. If the chain is "branched" the term ABS is applied.

c Alkyl sulfates comprise the third type of anionic surfactant.

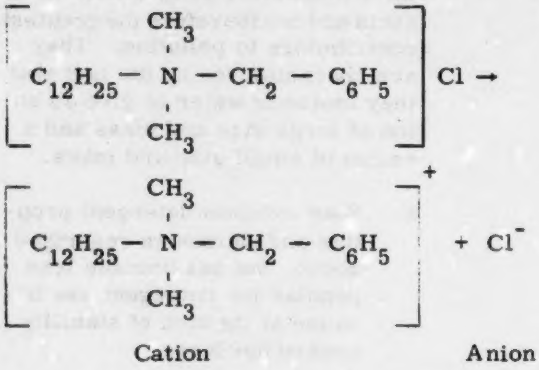
$$R\text{-}OSO_3Na \rightarrow R\text{-}OSO_3^- + Na^+$$

(Alkyl sulfate) (Anion) (Cation)

It again represents a chain of about twelve carbon atoms connected in straight line fashion.

2 **Cationic surfactants**

Compared to the anionic surfactants, the production of the cationics is small. They are noted for their germicidal properties and are used as sanitizing agents in connection with laundering and dishwashing. They ionize in water to give a cation of large size and mass and an anion of small size and mass.



$$\left[C_{12}H_{25} - \overset{CH_3}{\underset{CH_3}{N}} - CH_2 - C_6H_5 \right] Cl \rightarrow$$

$$\left[C_{12}H_{25} - \overset{CH_3}{\underset{CH_3}{N}} - CH_2 - C_6H_5 \right]^+ + Cl^-$$

Cation Anion

Interaction of cationic and anionic surfactants yields compounds which have neither germicidal nor detergent properties.

3 **Nonionic surfactants**

These do not ionize in water. They show little tendency to foam and are probably constituents of the so-called "controlled suds" detergents. Compounds, such as those illustrated, may have from 5 to 12 "ethoxy" or ether groups.

$$C_8H_{17}-C_6H_4-OC_2H_4(OC_2H_4)_n-OH$$

(A Nonionic Ether)

C **Physical Behavior of Anionic Surfactants**

One of the functions of a good detergent was stated to be its ability to link dirt or oil particles with the water (emulsifying action.) This linking ability can be considered by using an alkyl aryl sulfonate anion as an example.

$$R-C_6H_4-SO_3^-$$

(Alkyl Aryl Sulfonate Anion)

The $R-C_6H_4$ portion of the anion is referred to as being hydrophobic (water repelling); i.e., it is insoluble in water, but soluble in grease and oil. The SO_3 portion of the anion is hydrophilic (water attracting); i.e., it is soluble in water, but is insoluble in grease and oil. In Figure 1 below, the rod represents the hydrophobic portion of the alkyl aryl sulfonate anion and the ball represents the hydrophilic part of the anion. In dilute water solution the anion orient themselves around the dirt and grease particles as shown in Figure 2. The dirt and grease particles thus coated (emulsified) show little tendency to coagulate and settle.

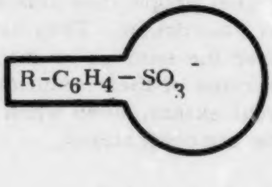


Figure 1.

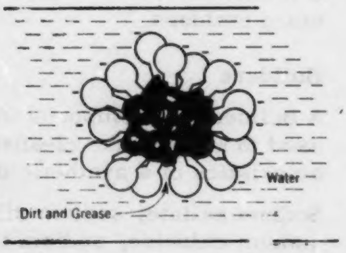


Figure 2. ACTION OF SURFACTANT ON DIRT PARTICLES(7)

II WASTES FROM THE USE OF SYNTHETIC DETERGENTS

A Sources of Wastes

1 Domestic wastes

The concentration of synthetic detergents (reported as MBAS) in raw sewage varies as shown in Table 1.

Table 1. MBAS CONTENT OF RAW SEWAGE (6)

City	MBAS in raw sewage (mg/l)
Oakland, California	4.4
Ponca City, Oklahoma	11.8
Cincinnati, Ohio	3.1
West New York, New Jersey	13.8

2 Laundry wastes

The average characteristics of a composite waste from a laundromat or small laundry operation, are presented in Table 2.

Table 2. LAUNDROMAT EFFLUENT (5)

COD (mg/l)	MBAS (mg/l)	pH (mg/l)	Suspended Solids (mg/l)
344 - 445	50 - 90	7.0 - 8.1	140 - 163

B Effects of Detergent Wastes on Water Quality

1 Foaming

MBAS levels in raw water at or below the USPHS Standards of 0.5 mg/l, do not cause foaming. At levels of 1 mg/l or above, foaming can occur.

Table 3 presents a summary of MBAS concentrations found in the Ohio River from 1954 to 1959, and reported by ORSANCO. (6)

Table 3. MBAS CONTENT OF OHIO RIVER WATER (1954 - 1959)

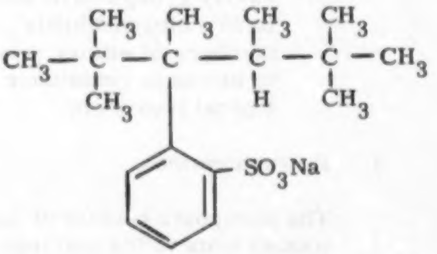
Value	MBAS (mg/l)
Median	0.12
Average	0.16
Weekly High	0.59
Weekly Low	0.01

2 Persistence in biological treatment

a Tetrapropylene ABS

Studies have indicated that branched ABS compounds made by alkylating benzene with tetrapropylene (see Figure 3) are only 40-70% biodegradable in conventional activated sludge treatment. Consequently, they are known as biologically "hard" compounds.

It has been hypothesized (4) that the structure of the $C_{12}H_{25}$ benzene sulfonate isomer, which would be most difficult to degrade, would be:



b Linear alkyl sulfonate (LAS)

On the contrary, compounds made by alkylating benzene with n-paraffins (see Figure 4) have shown up to 98% biodegradability in conventional activated sludge treatment. These biologically "soft" surfactants known as LAS (linear alkyl sulfonate) compounds,

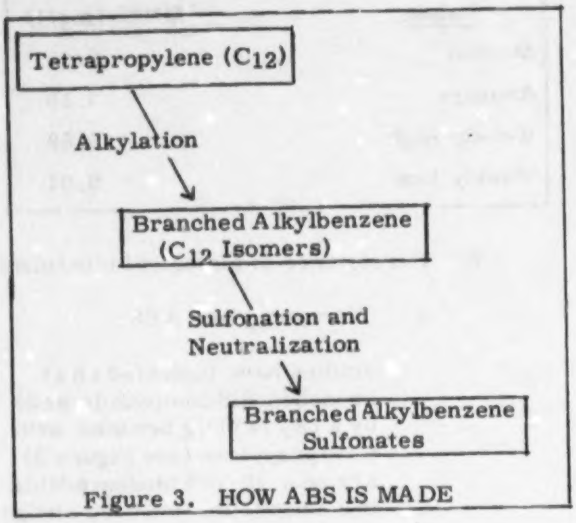

Tetrapropylene (C_{12})
Alkylation
Branched Alkylbenzene (C_{12} Isomers)
Sulfonation and Neutralization
Branched Alkylbenzene Sulfonates

Figure 3. HOW ABS IS MADE

have been developed commercially to replace biologically hard ABS compounds.

c Nonionics

Ethoxylated alkyl phoenol compounds containing less than 5 ethoxy groups have shown 90% to 95% biogradability. Larger numbers of ethoxy groups tend to increase resistance to biological treatment.

3 Eutrophication

The phosphate content of detergent wastes adds to the nutrient content of raw water. When the other essential nutrients are present, nuisance algal growth is promoted.

Compounds derived from amino carboxylic and hydroxycarboxylic acids are being considered as substitutes for the phosphate builders in order to decrease the nutrient load to streams and lakes.

III ANALYTICAL METHODS

A Sample Collection

If a sample cannot be analyzed promptly, several procedures may be followed in order to preserve the sample.

1 Freezing will retard biological activity.

2 The addition of 0.8 mg concentrated $H_2SO_4/1$ of sample will also retard biological activity and thus preserve the sample.

It should be noted that losses of MBAS' have been found to occur when samples are stored in polyethylene containers. (2) It is believed that these losses are due to adsorption of MBAS' on the sides of the containers.

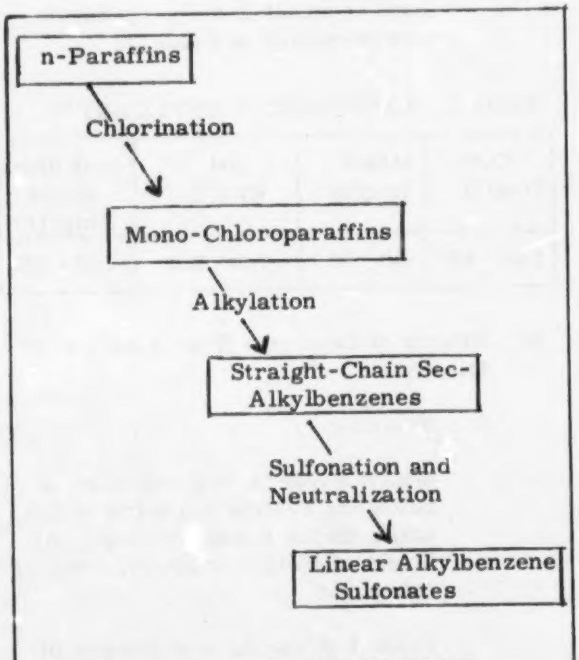

n-Paraffins
Chlorination
Mono-Chloroparaffins
Alkylation
Straight-Chain Sec-Alkylbenzenes
Sulfonation and Neutralization
Linear Alkylbenzene Sulfonates

Figure 4. HOW LAS IS MADE

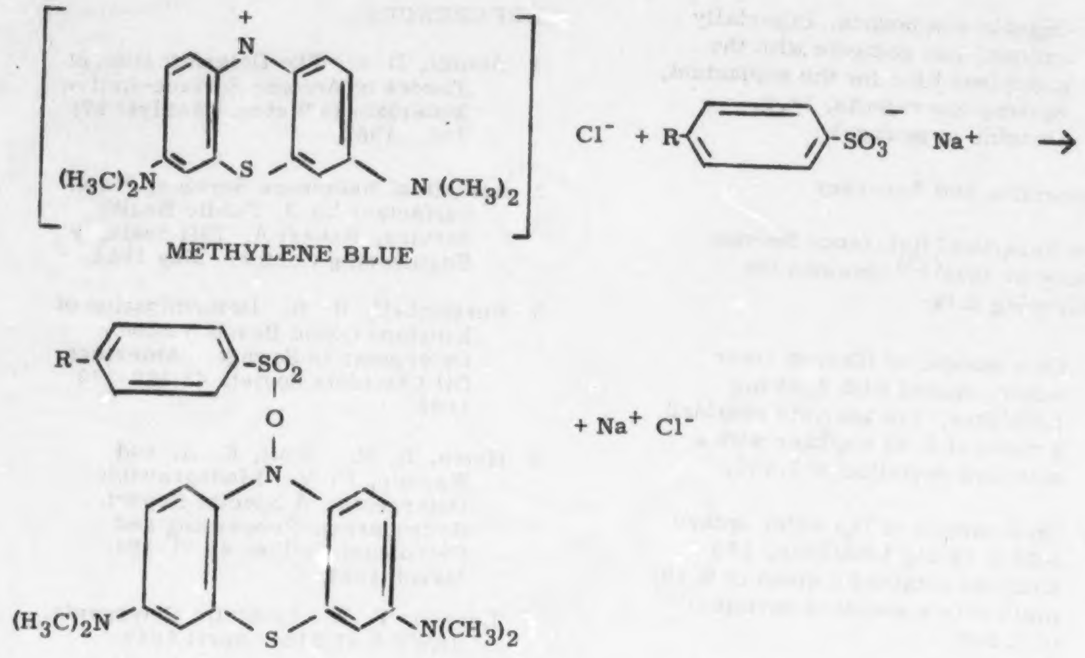


Figure 5. METHYLENE BLUE METHOD FOR ANIONIC SURFACTANTS$^{(7)}$

B Anionics - Methylene Blue Method

1 Principle

Methylene blue reacts with anionic surfactants (and other chemical species) to form a blue-colored, slightly ionized salt which is soluable in chloroform. The color intensity of this product in this solvent is measured at a wavelength of 652 nm in a spectrophotometer. Range of application is 0.025-100 mg/liter for LAS.

Surfactants and other chemical species which react with methylene blue are classed as methylene blue active substances (MBAS).

2 This is the method of analysis for MBAS listed in the current EPA Methods Manual (10). Reagent preparation and procedural details can be found in Standard Methods (11) and ASTM Book of Standards (12).

3 Interferences

a Glassware used in this method must be acid cleaned so that it is free of even a trace of surfactant material.

b Organic sulfates, sulfonates, carboxylates, phosphates and phenols will complex methylene blue, causing high results.

c Inorganic cyanates, chlorides, nitrates and thiocyanates form ion pairs with methylene blue, again causing high results.

d Organic compounds, especially amines, can compete with the methylene blue for the surfactant, causing low results. (e.g. proteins in sewage).

4 Precision and Accuracy

An Analytical Reference Service study in 1968(13) obtained the following data:

a On a sample of filtered river water, spiked with 2.94 mg LAS/liter, 110 analysts obtained a mean of 2.98 mg/liter with a standard deviation of 0.272.

b On a sample of tap water spiked with 0.48 mg LAS/liter, 110 analysts obtained a mean of 0.49 mg/l with a standard deviation of 0.048.

c On a sample of distilled water spiked with 0.27 mg LAS/liter, 110 analysts obtained a mean of 0.24 mg/l with a standard deviation of 0.036.

C Nonionics

1 Methods for nonionics have been based on formation of a Cobaltho-thiocyanate complex and subsequent colorimetric measurement. Burttschell(3) proposed a more sensitive method (0.1mg/l) in which a complex with the heterpoly acid of tungsten is formed, hydrolyzed, and colorimetrically measured as WO_4 dithiol.

2 Infrared method (3)

The Soap and Detergent Association has developed a referee method which measures "true" ABS or LAS, as opposed to "apparent" ABS or LAS, with the methylene blue method. This method consists of extensive clean-up procedures, followed by IR identification.

ACKNOWLEDGMENT

This outline contains certain portions of a previous outline by Betty Ann Punghorst, former Chemist, National Training Center.

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2 Analytical Reference Service, Water Surfactant No. 2, Public Health Service, Robert A. Taft Sanitary Engineering Center. May 1964.

3 Burttschell, R. H. Determination of Ethylene Oxide Based Nonionic Detergents in Sewage. American Oil Chemists Society 43:366-370. 1966.

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10 Methods for Chemical Analysis of Water & Wastes, U. S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

11 Standard Methods, 14th ed, 1975

REFERENCES (continued)

12 Annual Book of Standards, Part 31, Water, 1975.

13 Analytical Reference Service, Water Surfactant No. 3, Study No. 32, Public Health Service, Robert A. Taft Sanitary Engineering Center, 1968.

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, and revised by Audrey D. Kroner, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Chemical Analysis, Surfactants, Linear Alkylate, Sulfonates, Water Analysis

LABORATORY DETERMINATION OF SURFACTANTS
(Methylene Blue Active Substances, MBAS)

I REAGENTS

A Stock Linear Alkylate Sulfonate (LAS)

Weigh an amount of LAS (order from US EPA. EMSL. Quality Assurance Branch, Cincinnati. Ohio 45268) that will give a concentration of 1.00 mg LAS per 1.00 ml when dissolved in distilled water and diluted to 1 liter. Store the solution in a refrigerator. Prepare fresh weekly.

B Standard Linear Alkylate Sulfonate (LAS)

Dilute 10.0 ml of the stock LAS solution to 1 liter; 1.00 ml = 10.0 μg LAS. Prepare fresh daily.

C Phenolphthalein Indicator

D Sodium Hydroxide, 1 N

Dissolve 40.0 g of NaOH in water and dilute to 1 liter.

E Sulfuric Acid, 1 N

Slowly add 28.0 ml of concentrated H_2SO_4 to water and dilute to 1 liter.

F Chloroform, $CHCl_3$

G Methylene Blue

Dissolve 100 mg of methylene blue in 100 ml of distilled water. Transfer 30 ml of the solution to a 1 liter volumetric flask. Add 500 ml distilled water, 6.8 ml concentrated H_2SO_4, and 50 g $NaH_2PO_4 \cdot H_2O$. Shake the flask to dissolve the solid. Dilute to the 1 liter mark.

H Wash Solution

Add 6.8 ml concentrated H_2SO_4 to 500 ml of distilled water in a 1 liter volumetric flask. Add 50 g $NaH_2PO_4 \cdot H_2O$ and shake until the solid dissolves. Dilute to the 1 liter mark.

II PROCEDURE

A Pipet the following volumes of the standard LAS into 200 ml separatory funnels (with Teflon stopcocks): 0.0, 1.0, 3.0, 5.0, 7.0, 9.0, 11.0, 13.0, 15.0 and 20.0. Add sufficient water (graduated cylinder) to bring the volume in each flask to 100 ml.

B Add 100 ml of sample (graduated cylinder) to another separatory funnel.

C Add 3 drops of phenolphthalein indicator to the sample and standards, and mix.

D Add 1 N NaOH dropwise to the sample and standards until a permanent pink color is present.

E Discharge the pink color with 1 N H_2SO_4.

F Add 10 ml $CHCl_3$ and 25 ml of the methylene blue reagent (graduated cylinders) to the separatory funnels containing the sample and standards.

G Rock the funnels gently for a few seconds and relieve the pressure in the funnels.

H Rock the funnels vigorously for 30 seconds.

I Allow the layers to separate. The upper aqueous layer is dark blue in color while the lower $CHCl_3$ layer is light blue in color.

J Remove the funnel stoppers and drain the lower $CHCl_3$ layer into 125 ml Erlenmeyer flasks.

K Add 10 ml $CHCl_3$ to each of the funnels (sample and standards).

L Repeat steps G, H, I and J, using the same 125 ml Erlenmeyer flasks as in step J.

M Repeat the extraction process 2 more times (4 total) with 10 ml portions of $CHCl_3$. Note that in the case of the sample funnel, if the blue color disappears from the water layer during any of the 4 extractions, a smaller sample size must be used.

N Discard the water layers left in the sample and standards funnels.

O Rinse all of the separatory funnels vigorously with tap water, and then rinse with distilled water.

P Pour the $CHCl_3$ from the Erlenmeyer flasks back into the separatory funnels.

Q Add 50 ml of wash solution to the sample and standards funnels.

R Repeat steps G and H.

S Allow the layers to separate.

T Place a small piece of glass wool in a small filtering funnel.

U Place the filtering funnel in a 100 ml volumetric flask.

V Drain the lower $CHCl_3$ layer from the separatory funnel, through the glass wool in the filtering funnel, and into the 100 ml volumetric flask.

W Add 10 ml $CHCl_3$ to the separatory funnel containing the wash solution.

X Repeat steps G and H.

Y Allow the layers to separate.

Z Repeat step V.

AA Repeat the extraction with a second 10 ml portion of $CHCl_3$ and drain it into the 100 ml volumetric flask.

BB Rinse the filtering funnel and glass wool with a small amount of $CHCl_3$. Collect the rinsings in the 100 ml volumetric flask.

CC Bring the $CHCl_3$ in the volumetric flask to the 100 ml mark with $CHCl_3$.

DD Using a $CHCl_3$ blank and 1 cm cell, read the absorbance of the sample and standards at 652 nm in a Spectronic 20 spectrophotometer.

III CALCULATIONS

A Prepare a calibration graph for the standards of LAS concentration vs. absorbancy.

Example:

1 Concentration of standard LAS = 10.0µg LAS per 1.0 ml

2 $\frac{10\ \mu g\ \text{LAS}}{1.0\ \text{ml}} \times 5.0\ \text{ml of standard} = 50.0\ \mu g\ \text{LAS}$

3 The 50.0 µg may be used as a "mass" value on the X axis of the graph, or

4 The 50.0 µg is in a 100 ml volumetric flask.

$$\frac{50.0\ \mu g}{100\ \text{ml}} = \frac{500.0\ \mu g}{1000\ \text{ml}} = \frac{0.5\ \text{mg}}{\text{liter}}$$

The 0.5 mg per liter may be used as a "concentration" value on the X axis of the graph.

REFERENCE

Standard Methods for the Examination of Water and Wastewater, 13th ed., page 339, Method 159 A. American Public Health Association, American Water Works Association, Water Pollution Control Federation. Washington, D. C. 1971.

Standard Methods, 14th ed, Method 512A, pg 600, 1975.

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Surfactants, Laboratory Tests, Linear Alkylate, Sulfonates, Water Analysis

DATA SHEET

ml of LAS standard solution used	mg LAS/1	Absorbance
0.0		0.
1.0		0.
3.0		0.
5.0		0.
7.0		0.
9.0		0.
11.0		0.
13.0		0.
15.0		0.
20.0		0.

Absorbance of sample = 0. ______________

Concentration of LAS in sample in mg/liter from curve = ______________

OIL AND GREASE

I DEFINITION

A Definition

In the field of wastewater treatment, the terms oil and grease are not clearly defined. They are partially characterized by the analytical method used for their determination. A sample is extracted with an organic solvent which is then separated from the water and evaporated. The residue is termed oil and grease, regardless of its composition. Low boiling components, such as lubricating oil and kerosine, are lost, to some extent, during the solvent removal step. The determination of gasoline by the solvent extraction method is completely unreliable. Thus, the term oil and grease is operationally defined.

B National Pollutant Discharge Elimination System (NPDES)

Under provisions of the 1972 Amendments to the Federal Water Pollution Control Act (Public Law 92-500), the NPDES places limitations on the concentrations of pollutants which may be discharged to receiving bodies of water. One such pollutant is termed oil and grease. Thus, although the term is not clearly defined in the area of wastewater treatment, it does have significance under the NPDES.

C Components

In wastewater, the term grease includes such classes of compounds as waxes, fatty acids, fats, and oils. Classes of compounds referred to as oils are low to high molecular weight hydrocarbons, such as gasoline, heavy fuel oils and lubricating oils, and animal and vegetable glycerides which are liquid at ordinary temperatures.

II OCCURRENCE

Materials classified as oils and greases enter receiving bodies of water and wastewater treatment plants from households and industries which either manufacture or use the groups of compounds mentioned above. Examples of such industries are meat processing plants, petroleum refineries, petrochemicals, trucking, laundry, machine tool and steel.

III TREATMENT PROBLEMS

A Oil and grease cause special problems in the handling of household and certain industrial wastes, because they have a low solubility in water and therefore tend to separate from the water phase.

B They form scum layers in primary settling tanks, sludge digestion units, and final clarifiers. They coat particles, producing floating masses which are unsightly and odorous. When oil and grease coat organic particles, oxygen transfer and biodegradation are inhibited. Such interference can occur in the activated sludge process, as well as in trickling filters. In activated sludge plants, high oil/grease concentrations can result in significant carryover of biological solids during final clarification by entrapment of biofloc in the floating scum layer. Oil and grease are resistant to both aerobic biodegredation and anaerobic digestion. These materials cling to equipment surfaces such as pipelines, pumps, screens, and filters, thus reducing their operating efficiency. Also, they are a safety hazard in wastewater treatment plants, coating walkways and ladders. Grease particles are often present in an emulsified form. The emulsifying coating is sometimes not broken until the grease enters secondary treatment units or the receiving stream.

IV WATER SUPPLY PROBLEMS

Even small quantities of oil and grease can produce an objectional odor and appearance in public water supplies. If these materials are found in water contemplated for use as a public water supply, the source may be rejected, even before a health problem is shown to exist.

V ANALYTICAL METHODOLOGY

A NPDES

The analyses for pollutants performed under the NPDES (see I. B. above) are to be performed according to specified methodology. This methodology is spelled out in the Federal Register, Wed., Dec., 1, 1976, vol. 41, no. 232, pt II, pgs 52780-52786. The Federal Register cites the Freon extraction on pg 515 of the 14th ed. of Standard Methods (1), & an almost identical procedure on pg 229 of the EPA methods manual(2) as the procedures to be used when analyzing a wastewater sample for oil and grease. Steps in the analysis include: acidification of the sample with 5 ml of 50% by volume sulfuric acid per liter of sample, extraction of the sample with several portions of Freon (trichlorotrifluoroethane, boiling point 47°C; Dupont Freon precision cleaning agent or equivalent), combining the Freon portions in a tared distilling flask, distilling off all but about 10 ml of the Freon, boiling off the remaining Freon, drying the flask, cooling and weighing. The milligram increase in weight, multiplied by 1000, and divided by the milliliters of sample, gives the milligrams of oil and grease per liter of sample.

B Other Analytical Procedures, non NPDES

1. Standard Methods (1)

In addition to the above "approved" method, 14th Standard Methods (1) also carries two other procedures applicable to wastewater. The first is tentative, and involves an extraction identical to that described above, followed by infrared detection. The second utilizes Freon in a Soxhlet extractor. An eighty cycle extraction is followed by evaporation of the solvent and weighing of the residue.

2. U.S. EPA Methods Manual

In addition to the above "approved methods the U.S. EPA methods manual (2) carries two other procedures. In the first, the acidified sample is filtered through a muslin cloth disc overlaid with filter paper; filter aid is also used. The filter paper any any solids clinging to the muslin are then extracted in a Soxhlet apparatus with hexane. The solvent is evaporated and the increase in flask weight is used to calculate the mg of oil/grease per liter of sample. The second is an infrared method very similar to that given in 14th Standard Methods (1).

3 ASTM (3) lists no parameter specifically referred to as oil and grease.

C Sample Collection and Storage

The method referred to in V.A. above, directs that the sample must be representative. However, since oil and grease will be found on the surface of a body of water, the sample will not be representative of the body of water as a whole. The glass stoppered sample bottle should be washed with solvent and air dried. It should also be marked on the outside, to indicate the desired sample volume. None of the oil and grease should be lost by clinging to the glass stopper. Therefore, the bottle should not be filled to the top. Preservation is accomplished by adding 5 ml of 50% by volume sulfuric acid per liter of sample. No holding time is specified, but it is generally good procedure to begin the analysis as soon as possible.

REFERENCES

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2 Methods for Chemical Analysis of Water & Wastes, U. S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

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REFERENCES (CONTINUED)

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This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Chemical Analysis, Oil, Oil Pollution, Oil Wastes, Oily Water, Water Analysis

LABORATORY DETERMINATION OF OIL AND GREASE

I REAGENTS

A Freon TF (E. I. DuPont de Nemours) or Genosolv D (Allied Chemical Co.); the symbol TF/D will be used throughout this procedure to mean this particular solvent.

B Hydrochloric or Sulfuric Acid

Fifty percent by volume.

C Sodium Sulfate

II PROCEDURE

A Weigh a 125 ml distilling flask on an analytical balance. Handle the flask throughout the entire procedure with a tissue or crucible tongs. Set the flask aside until it is needed.

B Shake the sample container.

C Measure 1 liter of sample in a graduated cylinder.

D Measure 5 ml of 50% by volume HCl or H_2SO_4 in a small graduated cylinder.

E Add it to the 1 liter graduated cylinder.

Note: Do not do steps D and E if the sample was acidified at the time of collection. The sample pH should be 2, before doing step F. Check the sample with pH sensitive paper.

F Empty the 1 liter cylinder into a 1 liter separatory funnel (Teflon stopcock).

G Stopper the funnel and shake gently so as to mix the acid and sample.

H Check the pH of the sample with pH sensitive paper. If it is not 2 or less, add a few more drops of acid, mix, and recheck the pH.

I Rinse the 1 liter cylinder with 30 ml of TF/D (measured in a small graduated cylinder).

J Add it to the separatory funnel. The TF/D will form a separate layer beneath the water.

K Replace the funnel stopper, invert the funnel, and open the stopcock to relieve the pressure.

L Close the stopcock and shake the funnel gently for a few seconds.

M Open the stopcock to relieve the pressure.

N Repeat steps L and M.

O Close the stopcock and shake the funnel more vigorously for 2 minutes. Thorough mixing without excessive foaming is the objective.

P Repeat step M.

Q Place the funnel back in the ring stand and remove the stopper.

R Allow the water and TF/D layers to separate.

S Drain the lower TF/D layer into the previously weighed 125 ml distilling flask. About 1 drop of the TF/D should remain in the separatory funnel. If the TF/D layer is not clear, filter it into the flask through a small funnel containing filter paper and about 1 g (estimate) of anhydrous Na_2SO_4.

T Measure 30 ml of TF/D in a small graduated cylinder and use it to rinse the 1 liter graduated cylinder.

U Add the rinsings to the separatory funnel.

V Repeat the two gentle shakings, pressure relief, 2 minute vigorous shaking, and layer separation as above.

W Repeat step S, using the same distilling flask. The flask now contains about 60 ml of TF/D.

X Repeat steps T, U, V, and W. The flask now contains about 90 ml of TF/D. If the small funnel and Na_2SO_4 were used, wash them with a few ml of TF/D. Collect the washings in the distilling flask.

Y Evaporate the TF/D at 70°C on a water bath. (Heat the lower third of the flask only.)

Z Raise the temperature of the bath to 80°C for 15 minutes.

AA Apply suction to the warm flask for 1 minute. (See figure below).

BB Wipe the outside of the flask thoroughly with tissues and cool it in a desiccator for about 20 minutes.

CC Weigh the flask on the same balance as before.

III BLANK DETERMINATION

A To a second, weighed, 125 ml distilling flask, add about the same volume of TF/D which was used for the sample. This will be 90 ml, plus rinsings. The volume used for rinses will have to be estimated. Handle the flask in the same manner as described in II A.

B Repeat steps Y through CC, using the second flask.

IV CALCULATIONS

mg of oil and grease/liter =

$[A - B] - [C - D] \times 1000 \times 1000/\text{ml of sample}$

A = weight of sample flask + oil and grease, in grams

B = weight of empty sample flask, in grams.

C = weight of "blank" flask + residue, in grams.

D = weight of empty "blank" flask, in grams.

1000 = Conversion factor, grams to mg

1000 = Conversion factor, ml to liter

REFERENCE

Methods for Chemical Analysis of Water & Wastes, U. S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974.

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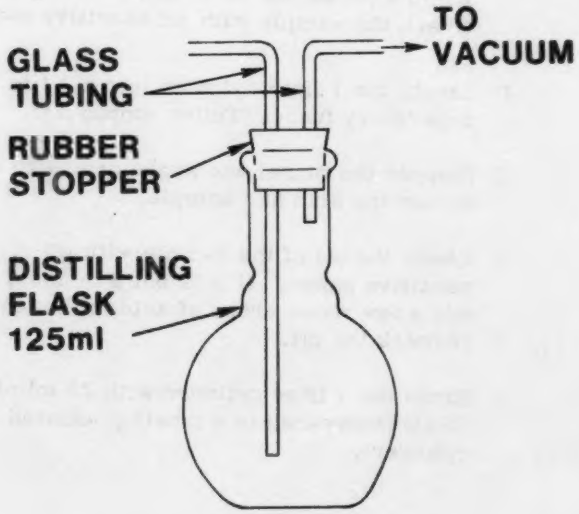
Descriptors: Chemical Analysis, Laboratory Tests, Oil, Oil Pollution, Water Analysis



DATA SHEET

sample identification =

ml of sample =

A = grams

B = grams

C = grams

D = grams

DETERMINATION OF PHENOLICS

I DEFINITION AND SIGNIFICANCE

A Definition

The phenolic compounds in water chemistry are defined as hydroxy derivatives of benzene and its condensed nuclei. These occur in domestic and industrial wastewaters and in drinking water supplies.

B Phenol and chlorinated derivatives in water affect fish and water quality.

1 Fish

The threshold limit of toxicity at infinite time for certain species of fish is of the order of a few milligrams per liter. Some chlorinated phenols exhibit toxicity in concentrations as low as 0.2 mg/l.

2 Fish flesh tainting

Fish living in waters of lesser phenolic concentrations can acquire an unpleasant and obnoxious taste.

3 Water quality

The presence of as little as 14 $\mu g/l$ of the chlorinated phenols can impart a taste to drinking water.

II CHLORINE DERIVATIVES OF PHENOL CAUSING TASTE AND ODOR(1)

A All chlorination products may contribute to the intensity of taste and odor.

B At maximum taste and odor intensity, the major contributor is 2, 6-dichlorophenol.

C The chlorine-to-phenol ratio at maximum intensity of taste and odor is 2:1. The proportion of 2, 6-DCP was greatest at the 2:1 chlorine-to-phenol ratio.

III PRESERVATION AND STORAGE OF SAMPLES

A Since phenolics are subject to oxidation, samples should be analyzed within 4 hours of collection.

B Samples can be preserved and stored up to 24 hours as follows:

1 Adjustment of pH to less than 4.0 with H_3PO_4

2 Aeration, if sulfides are present

3 Addition of 1.0 g $CuSO_4 \cdot 5H_2O$/liter

4 Storage at 4^oC

IV DETERMINATION OF PHENOLICS

A NPDES Methodology

The 1976 Federal Register Guidelines for National Pollutant Discharge Elimination System (NPDES) requirements specify distillation to separate out interferences, followed by the 4-amionoantipyrine (4AAP) colorimetric determination. Phenol is to be used as a standard.

Comments on the procedure can be found in the EPA Methods Manual.(2) The stepwise procedure can be found in Standard Methods(3) and ASTM(4).

B Other Analytical Procedures

For purposes other than NPDES requirements, Standard Methods(3) lists a 4AAP method for halogenated phenols which employs 2, 4-dichlorophenol as a standard. It also presents a gas-liquid chromatographic method for samples containing certain phenolics present in concentrations greater than 1 mg/liter.

Thin-layer chromatography has also been utilized for phenol and certain substituted phenols in raw surface water(5).

V PRETREATMENT AND DISTILLATION OF SAMPLES(3, 4)

Depending on interferences present, samples must be treated prior to the color determination.

A Pretreatment of Samples

1 Oxidizing agents, as detected by the odor of chlorine or by the starch-iodide test, are removed immediately after sampling by adding an excess of ferrous sulfate or sodium arsenite.

2 Oils and tars in a sample may contain phenolics. An alkaline extraction to remove these is required prior to adding $CuSO_4$ and distillation.

3 If sulfur compounds are present, e.g. H_2S or SO_2, and the sample has not been preserved, acidify the sample to less than 4 with H_3PO_4, and aerate it briefly. (These treatments are part of the preservation procedure if the presence of sulfur compounds is known).

B Distillation

NPDES specifies a preliminary distillation to remove common interferences. The rate of volatilization of phenols is gradual so the volume of the distillate should equal that of the sample being distilled. If the sample was not preserved, acidify it with 1 + 9 H_3PO4 and add copper sulfate solution.

1 Phenols are distilled from non-volatile impurities.

2 Addition of copper sulfate to sample forms CuS, thus preventing the formation of H_2S or SO_2 which interfere with the determination. $CuSO_4$ also prevents biochemical degradation of phenolics.

3 Acidification of the sample with phosphoric acid assures the presence of the copper ion and prevents the formation of $Cu(OH)_2$, an oxidizer of phenolics.

VI 4-AMINOANTIPYRINE DETERMINATION

A Applicability(3, 4)

This method determines phenol, ortho and meta-substituted phenols, and para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group. It does not determine those para-substituted phenols in which the substitution is an alkyl, aryl, nitro, benzoyl, nitroso or aldehyde group. Paracresol is an example of a common phenolic that is not sensitive to this determination.

B Method(2)

After pretreatment and distillation, the sample is reacted with 4-aminoantipyrine at pH 10.0 ± 0.2 in the presence of potassium ferricyanide (an oxidant) to produce colored antipyrine dyes. (See Figure 1).

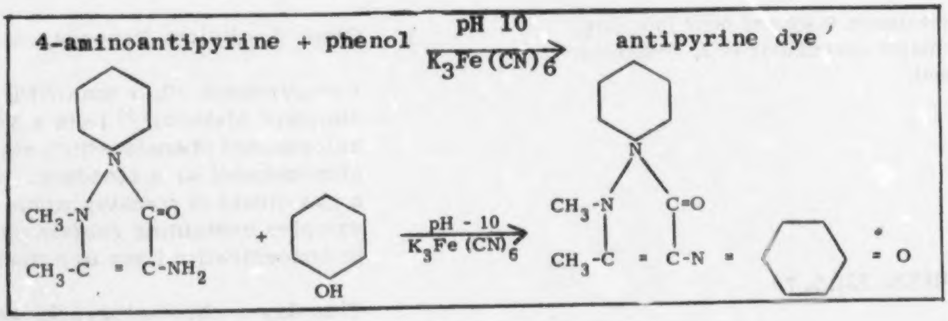


Figure 1

An absorbance measurement is made and the phenolic concentration is estimated using a calibration curve with phenol as a standard.

1 For original phenolic concentrations of 5μ g/l to 1000μ g/l, the reaction product dyes are concentrated using the Chloroform Extraction Method. Cell path lengths of 5cm or more are required for very low levels. Absorbance is measured at 460 nm, and the results expressed as μ g/liter phenol.

2 For original phenolic concentrations greater than 50μ g/liter, the reaction product dyes are kept in the water solution for a Direct Photometric determination. Absorbance is measured at 510nm., and the results are expressed as mg/liter phenol. This method is applicable to original phenolic concentrations up to 50 mg/liter.

3 Details of reagent preparation and the stepwise procedures can be found in the current editions of Standard Methods(3) and ASTM Standards (4).

C Variables

1 Sensitivity varies with pH. A buffer is used to maintain pH at 10.0 ± 0.2 to prevent the formation of antipyrine red and to minimize interference from aniline and undesirable enol-keto systems. (6, 7, 8)

The EPA manual notes that the ammonium hydroxide-ammonium chloride buffer used in the water hardness test is an alternative to the chemicals used in the other write-ups to raise the pH to 10 ± 0.2.

2 The amounts of 4-aminoantipyrine and potassium ferricyanide used have a definite bearing on the amount of color developed. (7)

3 Temperature affects the rate of color change of the product dyes and of the blank. All materials used should be at the same temperature. (6, 8)

4 Direct sunlight or strong artificial light may have a bleaching effect on the colored materials. (9)

5 Filtration of the chloroform extracts removes water and increases their color stability to 3 hours. The mixtures measured in water solutions are not too stable and should be read within 30 minutes. (6)

VII PHENOL STANDARD

1 Because phenol is extremely sensitive to the 4-aminoantipyrine determination, the calibration curve used in the procedure is derived using phenol standards. These standards are prepared on the day of use by diluting a more concentrated stock solution of phenol.

The stock solution of phenol can be prepared by direct weighing. If extreme accuracy is required, this stock solution can be standardized using a bromate-bromide solution. (3)

2 Phenolics (substituted phenols) respond with various sensitivities to this test and produce colors of various densities. An example is this comparison of the absorbance values of the cresols to that of phenol: (7)

Compound	Absorbance Values Compared to Phenol(%)
phenol	100
orthocresol	74
metacresol	69
paracresol	3

3 Most phenolics sensitive to the test produce dyes with absorbancy maxima at or near the same wavelength so a photometric determination can be made.

VIII ACCURACY (3, 7)

Results are an approximation and represent the minimum amount of phenol and phenolics present.

1 Only phenol is used as the color standard since it is impractical to prepare a standard containing a mixture of phenol and phenolics corresponding to each sample.

2 Different phenolics exhibit different sensitivities to the test.

3 Different phenolics produce differing shades of color which affect the final absorbance reading for the mixture.

IX PRECISION(2)

Reproducibility of results depends on on the interferences present in samples and on the skill of the analyst.

1 Using the Chloroform Extraction Method to concentrate color, six laboratories analyzed samples at concentrations of 9.6, 48.3 and 93.5 μg/liter. Standard deviation respectively, was 0.99, 3.1 and 4.2 μg/liter.

2 Using the Direct Photometric Method, six laboratories analyzed samples at concentrations of 4.7, 48.2 and 97.0 mg/liter. Standard deviation, respectively, was 0.18, 0.48, and 1.58 mg/liter.

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Descriptors: Chemical Analysis, Phenols, Water Analysis

LABORATORY DETERMINATION OF PHENOL
DIRECT PHOTOMETRIC METHOD

I REAGENTS

A Methyl Orange Indicator

Dissolve 0.5 g of the indicator in 1 liter of distilled water.

B Phosphoric Acid Solution

Dilute 10 ml of 85% H_3PO_4 to 100 ml with distilled water.

C Copper Sulfate Solution

Dissolve 100 g of $CuSO_4 \cdot 5H_2O$ in distilled water and dilute to 1 liter.

D Stock Phenol Solution

Dissolve 1.00 g of reagent grade phenol in freshly boiled and cooled distilled water and dilute to 1 liter. Ordinarily, this direct weighing of phenol constitutes a standard solution. However, if extreme accuracy is needed, the solution must be standardized [see section 510 B.3.a. 1) on page 578 of reference 2].

E Intermediate Phenol Solution

Dilute 10.0 ml of the stock phenol solution to 1 liter with freshly boiled and cooled distilled water;

1 ml = 10.0 μg phenol

F Ammonium Chloride Solution

Dissolve 50 g of NH_4Cl in distilled water and dilute to 1 liter.

G Aminoantipyrine Solution

Dissolve 2.0 g of 4-aminoantipyrine in distilled water and dilute to 100 ml. Prepare this solution on the day of use.

H Potassium Ferricyanide Solution

Dissolve 8.0 g of $K_3Fe(CN)_6$ in distilled water and dilute to 100 ml. Prepare this solution fresh weekly.

I Concentrated Ammonium Hydroxide

J pH 9, 10, or 11 buffer

II PROCEDURE

A Measure 500 ml of sample (graduated cylinder) and pour it into a large beaker.

B Add 3 drops of methyl orange indicator to the sample and mix.

C Add a drop of H_3PO_4 solution (eyedropper) to the sample and mix. A pink/red color should be present (pH approximately 4). If it is not, add a second drop of H_3PO_4 and mix.

D Pipet 5.0 ml of $CuSO_4$ solution into the sample and mix.

E Remove the small rubber stopper from the distilling apparatus, insert a small funnel in the hole, and pour the sample through the funnel into the distilling flask. Glass beads are already in the flask.

F Distill 450 ml of sample into a 1 liter Erlenmeyer flask.

G While the sample is distilling (ckeck it every 5-10 minutes), pipet the following amounts of intermediate phenol solution into 100 ml volumetric flasks and dilute to the mark with distilled water: 0.0, 0.5, 2.0, 5.0, 10.0, 20.0, and 35.0 ml. Use these ml values as markings on the volumetric flasks.

H Remove the source of heat from under the distilling flask.

I When boiling ceases, add 50 ml of phenol free distilled water (graduated cylinder) to the distilling flask.

J Begin the distillation again, collecting a total of 500 ml in the 1 liter Erlenmeyer flask. (If the distillate is turbid, additional treatment is required. See Method 510 A, par. 4c and 4d in Standard Methods[2]).

K Pipet 25.0 ml of the distillate into a 100 ml volumetric flask and dilute to the mark with distilled water. Mark the flask 25.

L Fill a 100 ml volumetric flask to the mark with the distillate. Mark the flask 100.

M Put the same numbers on nine 125 ml Erlenmeyer flasks as were used on the nine volumetric flasks.

N Pipet 2.0 ml of ammonium chloride solution into each of the nine Erlenmeyer flasks.

O Pour the contents of each volumetric flask into the correspondingly marked Erlenmeyer flask. Mix by swirling the flasks.

P Calibrate a pH meter using pH 9, 10, or 11 buffer.

Q Using an eyedropper, add concentrated ammonium hydroxide to each Erlenmeyer flask until a pH of 10.0 ± 0.2 is obtained. Be careful to rinse the electrode thoroughly after each use.

R Turn on the Spectronic 20 (warm up).

S Pipet 2.0 ml of 4-aminoantipyrine into all of the Erlenmeyer flasks. Mix well by swirling.

T Pipet 2.0 ml of potassium ferricyanide into all of the Erlenmeyer flasks. Mix well by swirling.

U After 15 minutes measure the absorbance of all nine of the solutions at 510 nm. "Zero" the instrument against the solution in the "0" flask, i.e., the reagent blank.

III CALCULATIONS

A Prepare a calibration graph with absorbancies along the vertical axis and mg of phenol along the horizontal axis. For example·

Concentration of intermediate phenol solution = 10.0 μg/ml. If 35.0 ml of this solution is used, then 0.350 mg (350.0 μg) is the value on the horizontal axis.

B Determine the mg of phenol present in the distillate (sample) and "scale it up" to a per liter basis. For example:

If there were 0.10 mg in 100 ml of distillate, then the final answer is 1.0 mg of phenol/liter.

Note: The name of the parameter as listed in Table 1 of the Federal Register, Wednesday, December 1, 1976, vol. 41, number 232 is "Phenols" (item 96). However, positive results are also given by certain substituted phenols.

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This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Laboratory Tests, Water Analysis, Phenols

DATA SHEET

ml of intermediate phenol solution used	mg phenol/l	Absorbance
0.0	________	0. ______
0.5	________	0. ______
2.0	________	0. ______
5.0	________	0. ______
10.0	________	0. ______
20.0	________	0. ______
35.0	________	0. ______

Absorbance of sample = 0. ________

Concentration of phenol in sample in mg/liter from curve = ________

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

Part I

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)

2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104° C) and 4-Methylcyclohexan (B.P. 103° C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8° C) and Benzene (B.P. 80.2° C)	Chromosorb Newsletter (FF-104)

C Disadvantages

1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.

2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector.(See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

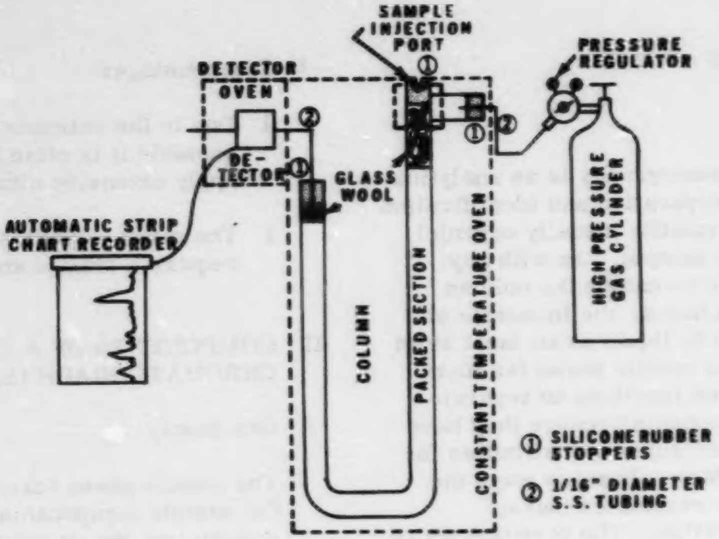
SAMPLE INJECTION PORT
PRESSURE REGULATOR
DETECTOR OVEN
DE-TECTOR
GLASS WOOL
AUTOMATIC STRIP CHART RECORDER
COLUMN
PACKED SECTION
CONSTANT TEMPERATURE OVEN
HIGH PRESSURE GAS CYLINDER
① SILICONE RUBBER STOPPERS
② 1/16" DIAMETER S.S. TUBING

Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH*

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m^2/gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B.P. 80.1°C) and cyclohexane (B.P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

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*Reproduced (with permission) from Chemistry. (37:11, p. 13. November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (picogram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture.

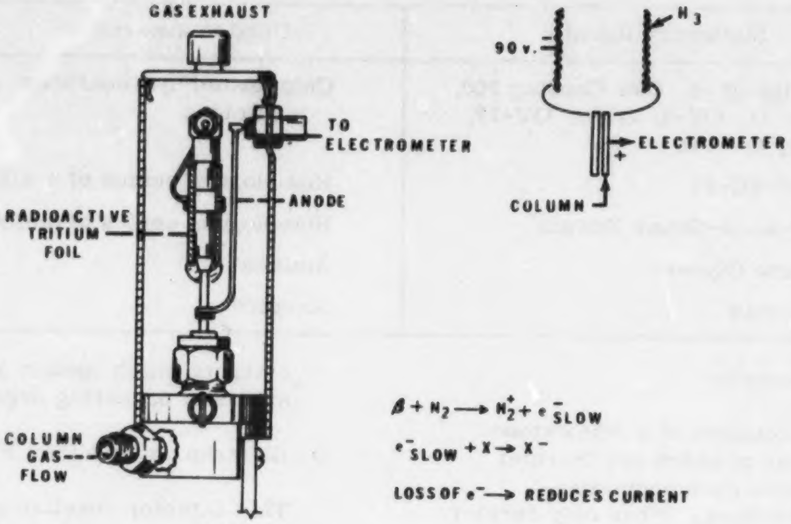
GAS EXHAUST
TO ELECTROMETER
ANODE
RADIOACTIVE TRITIUM FOIL
COLUMN GAS FLOW
90 v.
H3
ELECTROMETER
+
COLUMN
$\beta + N_2 \rightarrow N_2^+ + e^-_{SLOW}$
$e^-_{SLOW} + X \rightarrow X^-$
LOSS OF e^- → REDUCES CURRENT

Figure 2. ELECTRON CAPTURE DETECTOR (Wilkens Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO_2, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO_2 produced is continuously titrated by I_2 which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH_3 which is then titrated by H^+ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds.

The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

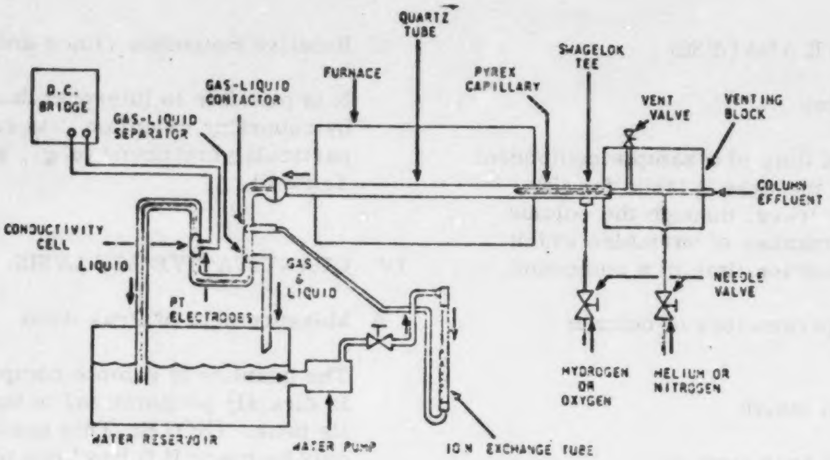
QUARTZ TUBE
FURNACE
GAS-LIQUID CONTACTOR
D.C. BRIDGE
GAS-LIQUID SEPARATOR
PYREX CAPILLARY
SWAGELOK TEE
VENT VALVE
VENTING BLOCK
COLUMN EFFLUENT
CONDUCTIVITY CELL
LIQUID
GAS & LIQUID
PT ELECTRODES
NEEDLE VALVE
HYDROGEN OR OXYGEN
HELIUM OR NITROGEN
WATER RESERVOIR
WATER PUMP
ION EXCHANGE TUBE

Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR (Coulson Instruments Company)

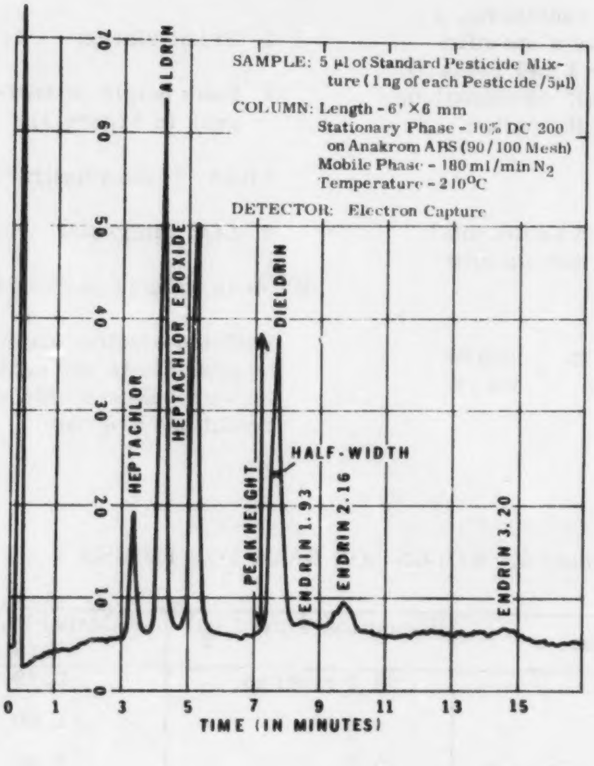

Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

1 Physical parameters of column operation

  a Column length

  b Column temperature

  c Carrier gas flow rate

2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME } (R_V) = \text{RETENTION TIME } (R_T) \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

1 Planimeter

2 Triangulation

3 Peak height × half-width (see dieldrin peak in Figure 3)

AREA = peak height × peak half-width

4 Disc integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R_T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

1 A steady basline is obtained.

2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

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Descriptors: Adsorbents, Chromatography, Gas Chromatography, Organic Matter, Separation Techniques, Water Analysis

QUANTITATION IN GAS CHROMATOGRAPHY

I INTRODUCTION

One of the factors which has led to gas chromatography's rapid development in the field of instrument analysis is the quantitative precision with which samples can be analyzed. There are several equally important factors involved in quantitative analysis. These are: Accurate sample introduction, constant operating parameters, accuracy of peak area measurement, sensitivity factors of individual compounds, linearity of detector and columns which give well resolved peaks.

II CALIBRATION PROCEDURE

A Peak Height and Peak Area

Quantitative work is based on peak height or area. Peak height measurement is more rapid than peak area; however, plots of peak height vs. sample size are more non-linear than corresponding plots for peak area. This is because peak heights and widths are frequently dependent on sample size and sample feed volume, however; total area is not. Peak heights are generally used if samples are less than 10 μg for packed columns and 0. 1 μg for capillary columns.

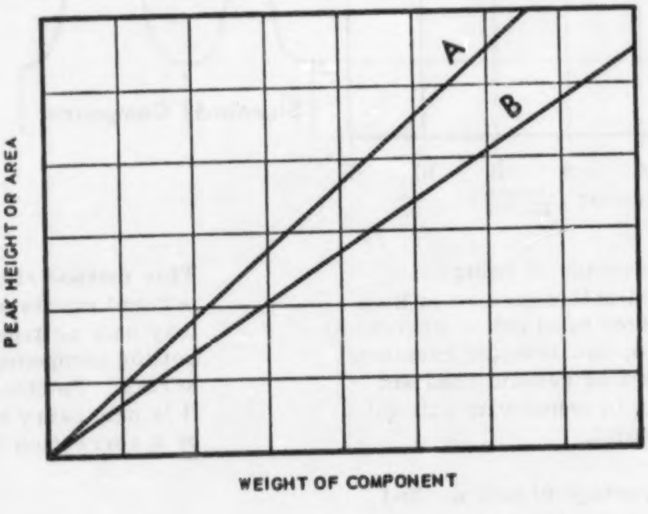
B Direct Calibration

Blends of components in question are prepared and chromatographed. The value of peak heights or areas are then plotted against the weight of sample injected. The unknown sample is then injected and its peak height or area is compared to that of the standard.

The main disadvantages of direct calibration are that the precise amount of sample injected must be known and that calibration is time consuming. Also, the sensitivity of the detector must remain constant from run to run and day to day in order to compare results with the calibration graph.

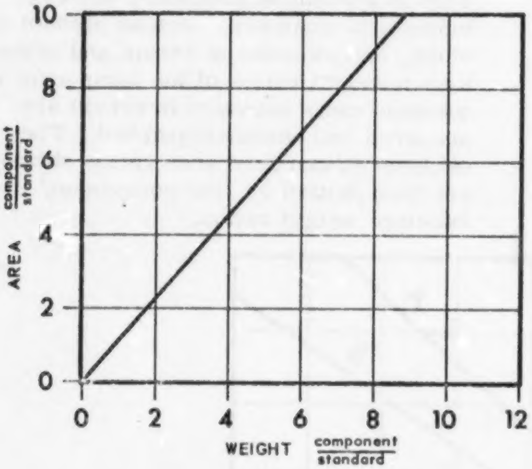
C Internal Standardization

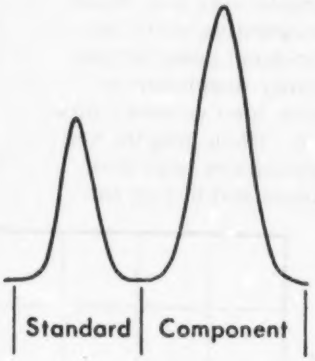
This is a common procedure used in biomedical analyses, such as alcohol in blood, and steroids in serum and urine. Known weight ratios of the component in question and a standard (marker) are prepared and chromatographed. The component/standard area ratios obtained are then plotted vs. the component/standard weight ratios.



To the unknown, add a known volume concentration of the standard. This mixture is then chromatographed and the component/standard area ratios are determined. By determining the corresponding component/standard weight ratio from the graph, the unknown amount of the component can be determined.

e. g.: To the unknown, add 5 ml of a solution containing the standard whose concentration is 100 μg/ml. Upon chromatographing the mixture, the area ratio was found to be 8; therefore, the weight ratio is 7 (see graph). Knowing standard concentration to be 100 μg/ml, then the component concentration is 7 × 100 μg/ml. Since we added 5 ml of standard solution, then the total amount of the unknown is 5 ml × 700 $\frac{\mu g}{ml}$ = 3500 μg or 3.5 mg.



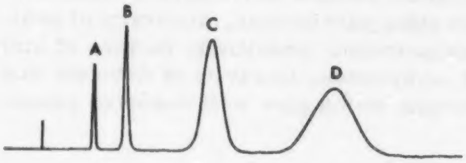


A particular advantage of using the internal standardization method is that the amount injected need not be accurately measured. Also, the detector response need not be known or remain constant since any change in sensitivity will not affect the area ratio.

The chief disadvantage of this method is the difficulty in finding a standard that does not interfere with a component in the sample. It is also time consuming.

D Internal Normalization

This is used when only approximate data are required. Assuming all the components have been eluted, the percent composition of a component within a mixture is its area divided by the sum of the areas.



$$\% \text{ Composition } A = 100 \left(\frac{A}{A+B+C+D}\right)$$

This method also assumes that area percent equals weight percent. This may only be true when analyzing close boiling components of a homologous series. To obtain the weight percent, it is necessary to multiply each area by a correction factor.

III NORMALIZATION OF AREAS (CORRECTION FACTORS)

A Normalization for Thermal Conductivity Detectors

This procedure relies on the fact that each compound has a unique thermal conductivity cell response. For quantitative analysis, the thermal conductivity of the sample should be approximately a linear function of its composition in the range concerned. From the areas under each peak and the relative responses that are characteristic of each peak, it is possible to determine the quantity of each component in the sample.

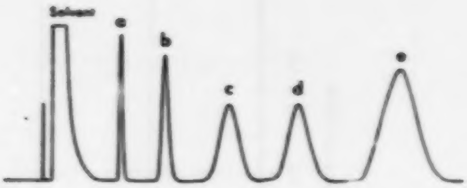
Sample calculation:

COMPOUND	AREA	RELATIVE T.C. RESPONSE PER MOLE (2)
Ethanol	5.0	72
Heptane	9.0	143
Benzene	4.0	100
Ethyl Acetate	7.0	111

			NORMALIZE		MOLE %
Ethanol	$\frac{5.0}{72}$	= 0.070	$\frac{0.070}{0.236}$	=	29.6
Heptane	$\frac{9.0}{143}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
Benzene	$\frac{4.0}{100}$	= 0.040	$\frac{0.040}{0.236}$	=	17.0
Ethyl Acetate	$\frac{7.0}{111}$	= 0.063	$\frac{0.063}{0.236}$	=	26.7
TOTAL		0.236			100.0%

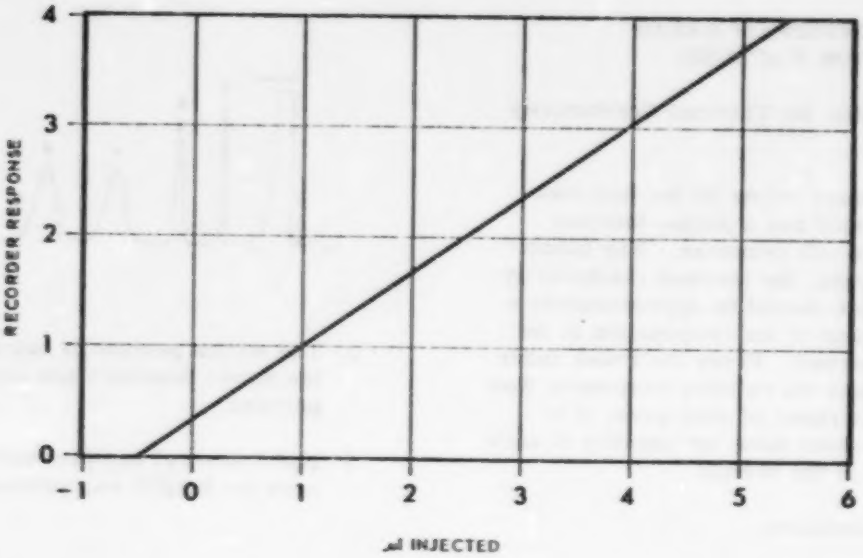
B Normalization for Mass Detectors

1 A standard solution of compounds a, b, c, d, and e in a solvent is prepared.



2 The weight percent is calculated and the known densities are used to convert percent.

3 Inject several samples and plot peak area (or height) vs. volume injected.



4 Note that intercept on "X" axis corresponds to an injection error of 0.5 µl. Therefore, actual amount injected = uncorrected + 0.5 µl. For 2.5 µl (corr.) injected, the density values are used to determine the actual weight W. injected.

5 The corresponding area of each peak is determined and the response in cm^2/µg is determined.

6 Using compound "a" as standard (correction factor = 1), the corresponding correction factors are determined. See the Table on Page 8-6.

IV STATISTICAL TREATMENT OF DATA

A Accuracy and Precision Data (Reproducibility)(3, 4)

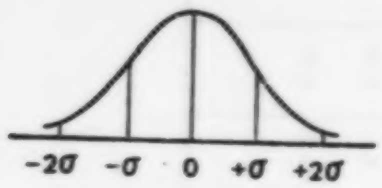
Accuracy is a measurement of the difference between the true value and the determined values. In those cases in which the true value is not known, it is necessary to express the exactness of a measurement in another way. This may be done by obtaining the average of a number of measurements and finding the difference (deviation) of each value from the average value. The magnitude of deviation is a measure of the precision of measurement.

It is seen that accuracy expresses the correctness of a measurement, whereas precision expresses the reproducibility of a measurement.

The accuracy and reproducibility achieved in gas chromatography depend on many things; among them, the correct choice of column, temperature, flow rate, sample size, detector, and injection system. Highest performance demands considerable understanding of the chromatographic process and of the effects of change in a large number of variables. However, even with ill-designed apparatus, analyses can usually be conducted with an accuracy better than ± 10 percent per component. When precautions are taken, an accuracy and reproducibility of about ± 1 percent per component is easily attainable.

B Errors

Errors fall into two classes. Determinate errors are those whose cause and magnitude can be determined. (Errors in method, apparatus, operator, etc.) Indeterminate errors are random errors which cannot be eliminated. The distribution of indeterminate errors follows the normal probability law as shown in the error curve. This curve shows that positive and negative deviations are equally probable and that small deviations occur much more frequently than large ones.



σ = Standard Deviation
$\pm\sigma$ = 68%
$\pm 2\sigma$ = 95%

V MEASUREMENT OF AREAS

A Methods

1 Cutting and weighing is a fairly accurate but tedious procedure. Besides the errors that may arise from thickness and moisture content of the paper, the chromatogram is destroyed. It is not satisfactory for estimating areas of overlapping peaks.

2 The planimeter is a devise whose acquisition is rarely justified. In addition to being very fatiguing, it is necessary to acquire considerable practice in its manipulation. The sensitivity of a normal planimeter is usually 10 mm^2, which is in many cases insufficient for analytical purposes.$^{(5)}$

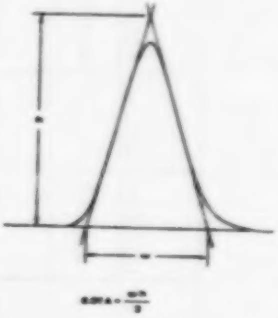
3 Integrators

a Mechanical integrators are exemplified by the Disc Integrator. This is a device which is attached to most strip chart recorders. The integrator pen tracing is displayed at the bottom of the chromatogram and the pen speed is proportional to the displacement of the recorder pen from baseline. (See triplicate runs of the nonanedecane mixture.) This is a frequently used, accurate device.

b Electronic integrators are devices which automatically print cumulative integrals of peak area. In addition to offering outstanding precision, electronic integrators automate chromatographic operations. This eliminates the time consuming necessity of the chemist keeping a watchful eye on the operation. Its biggest disadvantage is its cost.

4 Triangulation

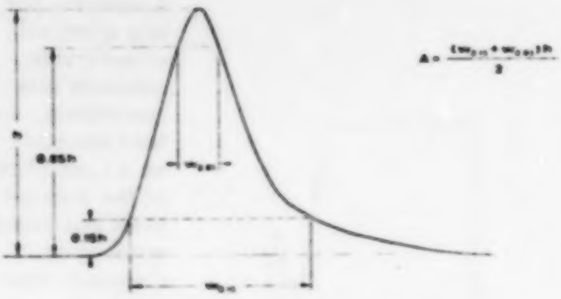
a Gaussian peaks



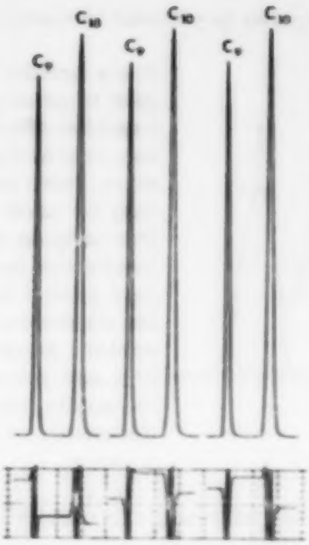
SAMPLE	WT. %	DENSITY	VOL. %	VOL. INJECTED, µl		W WT. INJECTED	A AREA	cm²/µg	F CORRECTION FACTOR
		gm/cm³		uncorr.	corr. (actual)	µg	cm²		
Solvent	99.79	.88	99.781	1.99562	2.49453	2195.19	--	--	--
a*	.02	0.50	.035	.00070	.00087	.435	4.0	9.19	1.00
b	.03	0.75	.035	.00070	.00087	.653	6.5	9.95	1.08
c	.04	0.90	.0387	.00077	.00096	.864	7.6	8.79	.96
d	.04	0.90	.0387	.00077	.00096	.864	8.1	9.38	1.02
e	.08	1.00	.0704	.00141	.00176	1.760	15.0	8.52	.93
Total	100.00		100.00	2.00	2.50	2199.766			

*Correction factors calculated relative to "a" as standard.

b Tailing or leading peaks, trapezoidal construction.



Triplicate analysis of nonane-decane mixture



B Comparison of Integration Methods

Using several of the above methods on the triplicate runs of the nonanedecane mixture, the following table was obtained:

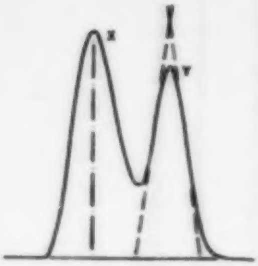
COMPARISON OF INTEGRATION METHODS

	#1	#2	#3	Avg.	σabs	σrel
MODEL 471 DIGITAL INTEGRATOR						
n-Nonane	39.156	39.150	39.193	39.166	0.0716	0.184%
n-Decane	60.844	60.850	60.807	60.834	0.0227	0.037%
DISC INTEGRATOR						
n-Nonane	39.33	38.97	38.91	39.07	0.22	0.56%
n-Decane	60.67	61.03	61.09	60.93	0.23	0.38%
TRIANGULATION						
n-Nonane	40.77	40.68	40.07	40.51	0.38	0.94%
n-Decane	59.23	59.32	59.93	59.49	0.39	0.66%
WEIGHING PAPER						
n-Nonane	42.58	41.73	42.83	42.38	0.58	1.37%
n-Decane	57.42	58.27	57.17	57.62	0.58	1.01%

Summary: Electronic digital integration is the most precise quantitation method; weighing paper is the least precise. Based upon this and other data in this publication, the electronic integrator give 2 to [illegible] times more precision than Disc Integration, 4 to 10 times more precision than triangulation, and 7 to 25 times more precision than cutting and weighing paper.

C Measurement of Overlapping Peaks

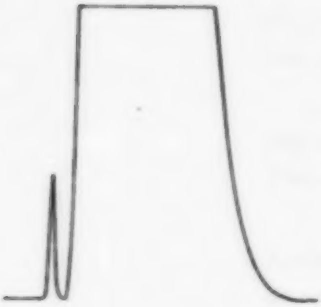
1 Components present in equal amounts.



Since broken line tangent to peak y intersects baseline after peak x has reached its maximum, then peak heights may be used. If broken line tangent to peak y intersects baseline before peak x has reached its maximum, then various known mixtures of x and y are prepared, chromatographed, and compared to the unknown x and y.

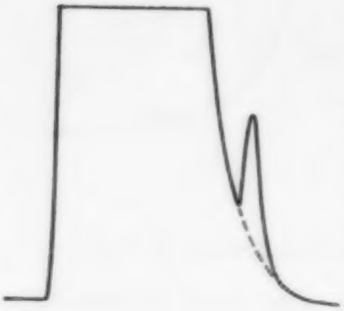
2 Trace analysis (less than 20 ppm)

a Trace component eluting before major peak.



In any trace analysis, one should choose a column and conditions which will allow the minor peak to elute before the major peak. This is easier to quantitate, and generally, peak height methods are used.

b Trace component on tail of major peak.



Assuming one has tried all conceivable columns and conditions, extend baseline as a continuation of the solvent peak and determine area. Compare this area with a synthetic mixture. Also, one can "spike" the sample with known amount of the trace component and compare area before and after.

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This outline was prepared by E. J. Bonelli, Applications Manager, Varian Aerograph Company, Walnut Creek, California.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Measurement, Separation Techniques, Water Analysis

POLYCHLORINATED BIPHENYLS

I INTRODUCTION

Polychlorinated Biphenyls (PCB's) have been widely discussed in the literature and news media in recent years. Although the topic levels have not been thoroughly evaluated, limits for concentrations in drinking water have been set at 100µg/l. Current studies have indicated PCB's to have a lower chronic toxicity than DDT by a factor of at least 2. Toxic levels for animal life vary considerably with species. However, due to the pronounced capacity of aquatic life to accumulate PCB's in the mg/l range and hence become toxic or killed from exposure to PCB's in the low µg/l range, a maximum allowable concentration of 25 mg/l has been recommended.

II OCCURRENCE

The widespread use of PCB's have resulted in the release into the environment of these materials. They have been found in rainwater, human tissue and many species of wildlife. Originally intended for industrial chemicals because of their nonflammability, high dielectric constant and plasticizing abilities, their use has grown steadily amounting to an estimated use of 400,000 tons in 1972 in the United States alone. Since the findings of PCB in the environment, the use of these materials have been reduced to about 20,000 tons/year. Sales of PCB's for all general plasticizer applications were discontinued on August 30, 1970, and are being phased out in other applications. These compounds are, like the chlorinated hydrocarbon pesticides such as DDT, very slow to degrade once they have entered the environment, and once ingested, are stored in the body's fatty tissue. The PCB's produced commercially are mixtures, incorporating some 50 or more of the 210 different PCB compounds. These mixtures are produced commercially in the United States solely by one company under the trade name of Arochlor.

III ANALYTICAL METHODOLOGY

The method of analysis for these compounds uses gas chromatography and is part of the National Pollutant Discharge Elimination System. The method can be found in the Federal Register, 38, No. 75, Part II. Because of the similarity in nature between the PCB and chlorinated pesticides, the same method can determine both. In fact, if both are present, they cause interferences in the identification of each other. Consequently, the analytical method contains a technique to separate the two.

Basically the method utilizes an extraction step with 15% methylene chloride in hexane and subsequent concentration. Then an initial run is made on a gas chromatograph to determine the complexity of the sample. If other interferences, or the degree of complexity, is too large, an additional clean up procedure must be carried out. Ultimately the PCB's are determined on the G.C. This determination is expressed as the Arochlor number after the basic chromatogram for the particular mixture has been identified. The method covers the determination of certain polychlorinated biphenyl mixtures including: Arochlors 1221, 1232, 1242, 1248, 1254, 1260, and 1016. The limit of detection is approximately 1 µg/l for each Arochlor mixture.

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This outline was prepared by J. D. Pfaff, Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Water Analysis, Polychlorinated Biphenyls, PCB, Arochlors, Chlorinated Hydrocarbon Pesticides, Pesticide Residues.

METHOD FOR POLYCHLORINATED BIPHENYLS (PCB'S) IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

A This method covers the determination of certain polychlorinated biphenyl (PCB) mixtures including: Aroclors 1221, 1232, 1242, 1248, 1254, 1260 and 1016.

B The method is an extension of the method for organochlorine pesticides in industrial effluents.(1) It is designed so that determination of both the PCB's and the organochlorine pesticides may be made on the same sample.

C The limit of detection is approximately 1 μg/l for each Aroclor mixture.

II SUMMARY

A The PCB's and the organochlorine pesticides are co-extracted by liquid-liquid extraction and, insofar as possible, the two classes of compounds separated from one another prior to gas chromatographic determination. A combination of the standard Florisil column cleanup procedure and a silica gel microcolumn separation procedure are employed.(2, 3) Identification is made from gas chromatographic patterns obtained through the use of two or more unlike columns. Detection and measurement is accomplished using an electron capture, microcoulometric, or electrolytic conductivity detector. Techniques for confirming qualitative identification are suggested.

III INTERFERENCES

A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to (4), Part I, Section 1.4 and 1.5.

B The interferences in industrial effluents are high and varied and pose great difficulty in obtaining accurate and precise measurement of PCB's and organochlorine pesticides. Separation and cleanup procedures are generally required to eliminate these interferences; however, such techniques may result in the loss of certain organochlorine compounds. For this reason great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial wastes.

C Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection. These materials do not interfere when the microcoulometric or electrolytic conductivity detectors are used in the halogen mode.

D Organochlorine pesticides and other halogenated compounds constitute interferences in the determination of PCB's. Most of these are separated by the method described below. However, certain compounds, if present in the sample, will occur with the PCB's. Included are: Sulfur, Heptachlor, aldrin, DDE, technical chlordane, mirex, and to some extent o,p'-DDT and p,p'-DDT.

IV APPARATUS AND MATERIALS

A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

  1 Electron Capture - Radioactive (tritium or nickel-63)

  2 Microcoulometric Titration

  3 Electrolytic Conductivity

C Recorder - Potentiometric strip chart (10 in.) compatible with detector system.

D Gas Chromatographic Column Materials:

  1 Tubing - Pyrex (180 cm long x 4 mm ID)

  2 Glass Wool - Silanized

  3 Solid Support - Gas-Chrom Q (100-120 mesh)

  4 Liquid Phases - Expressed as weight percent coated on solid support:

    a SE-30 or OV-1, 3%

    b OV-17, 1.5% + QF-1, 1.95%

E Kuderna-Danish (K-D) Glassware (Kontes)

  1 Snyder Columns - three ball (macro)

  2 Evaporate Flask - 500 ml

  3 Receiver Ampuls - 10 ml, graduated

  4 Ampul stoppers

F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - Pyrex (approximately 400 mm long x 20 mm ID) with a coarse fritted plate on bottom.

H Micro Column Pyrex - constructed according to Figure 1.

I Capillary pipets disposable (5-3/4 in.) with rubber bulb. (Scientific Products P5205-1).

J Low pressure regulator - 0 to 5 PSIG - with low-flow needle valve (See Figure 1, Matheson Model 70).

K Beaker - 100 ml

L Micro syringes - 10, 25, 50 and 100 μl.

M Separatory Funnels - 125 ml, 100 ml, and 2000 ml with Teflon stopcocks.

N Graduated Cylinders - 100 ml, 250 ml and 1000 ml.

O Blender - High Speed, glass or stainless cup.

P Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 in foil-covered glass container. Determine lauric-acid value (See Appendix I).

Q Silica gel - Davison code 950-08-08-226 (60/80 mesh).

R Glass Wool - Hexane extracted.

S Centrifuge Tubes - Pyrex calibrated (15 ml).

V REAGENTS, SOLVENTS AND STANDARDS

A Ferrous Sulfate - (ACS) 30% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

D Sodium Hydroxide - (ACS) 10 N in distilled water.

E Sodium Sulfate - (ACS) Granular, anhydrous, conditioned for 4 hours @ 400 C.

F Sulfuric Acid - (ACS) Mix equal volumes of concentrated H_2SO_4 with distilled water.

G Diethyl Ether - Nanograde, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

3 Distill deperoxidized ether in glass and add 2% ethanol.

H n-Hexane - Pesticide quality (NOT MIXED HEXANES).

I Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (Boiling range 30-60°C) - pesticide quality, redistill in glass if necessary.

J Standards - Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016*

K Anti-static Solution - STATNUL, Daystrom, Inc., Weston Instrument Division, Newark, N.J. 95212.

*May be ordered from US EPA, EMSL, Quality Assurance Branch, Cincinnati, Ohio 45268.

VI CALIBRATION

Gas chromatographic operating conditions are considered acceptable when the response to dicapthon is at least 50% of full scale when $\lesssim$ .06 ng is injected for electron capture detection and $\lesssim$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

B Standards are injected frequently as a check on the stability of operating conditions, detector and column. Example chromatograms are shown in Figures 3 through 8 and provide reference operating conditions.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as a quality control check. When the routine occurrence of a pollution parameter is observed, quality control charts are also recommended.(5)

B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equal to that used to dilute the sample.

VIII SAMPLE PREPARATION

A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

B For a sensitivity requirement of 1 μg/l, when using microoulometric or electrolytic conductivity methods for detection take 1000 ml of sample for analysis. If interferences pose no problem the sensitivity of the electron capture detector should permit as little as 100 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

C Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

IX EXTRACTION

A Add 60 ml of 15% methylene chloride in hexane ($v:v$) to the sample in the separatory funnel and shake vigorously for two minutes.

B Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pour the organic layer into a 100 ml beaker and then pass it through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

C Concentrate the extract to 6-10 ml in the K-D evaporator on a hot water bath.

D Qualitatively analyze the sample by gas chromatography with an electron capture detector. From the response obtained decide:

1 If there are any organochlorine pesticides present,

2 If there are any PCB's present,

3 If there is a combination of 1 and 2,

4 If elemental sulfur is present,

5 If the response is too complex to determine 1, 2, or 3.

6 If no response, concentrate to 1.0 ml or less, as required, according to EPA Method,(4) pg. 28 and repeat the analysis looking for 1, 2, 3, 4, and 5. Samples containing Aroclors with a low percentage of chlorine, eg. 1221 and 1232, may require this concentration in order to achieve the detection limit of 1 μg/1. Trace quantities of PCB's are often masked by background which usually occur in the samples.

E If condition 1 exists, quantitatively determine the organochlorine pesticides according to (1).

F If condition 2 exists, PCB's only are present, no further separation or cleanup is necessary. Quantitatively determine the PCB's according to XI below.

G If condition 3 exists, compare peaks obtained from the sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCB's from the organochlorine pesticides, continue as outlined in X D.

H If condition 4 exists separate the sulfur from the sample using the method outlined in (X C) followed by the method in (X E).

I If condition 5 exists then the following macro cleanup and separation procedures (X B and X C) should be employed and, if necessary, followed by the micro separation procedures (X D and X E).

X CLEANUP AND SEPARATION PROCEDURES

A Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as, the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether cleanup is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

B Acetonitrile Partition - This procedure is used to remove fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for the compounds of interest.

1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-35 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

5 Analyze by gas chromatography unless a need for further cleanup is indicated.

C Florisil Column Adsorption Chromatography

1 Adjust the sample extract volume to 10 ml.

2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition below). Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated on the following page:

6% Eluate

Aldrin
BHC
Chlordane
DDD
DDE
DDT
Heptachlor
Heptachlor Epoxide
Lindane
Methoxychlor
Mirex
Pentachloronitrobenzene
Strobane
Toxaphene
Trifluralin
PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual.(6)

4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

5 Analyze by gas chromatography.

D Silica Gel Micro-Column Separation Procedure (7)

1 Activation for Silica Gel

a Place about 20 gm of silica gel in a 100 ml beaker. Activate at 180 C for approximately 16 hours. Transfer the silica gel to a 100 ml glass stoppered bottle. When cool, cover with about 35 ml of 0.50% diethyl ether in benzene (volume:volume). Keep bottle well sealed. If silica gel collects on the ground glass surfaces, wash off with the above solvent before resealing. Always maintain an excess of the mixed solvent in bottle (approximately 1/2 in. above silica gel). Silica gel can be effectively stored in this manner for several days.

2 Preparation of the Chromatographic Column

a Pack the lower 2 mm ID Section of the microcolumn with glass wool. Permanently mark the column 120 mm above the glass wool. Using a clean rubber bulb from a disposable pipet seal the lower end of the microcolumn. Fill the microcolumn with 0.50% ether in benzene (*v:v*) to the bottom of the 10/30 joint (Figure 1). Using a disposable capillary pipet, transfer several aliquots of the silica get slurry into the microcolumn. After approximately 1 cm of silica gel collects in the bottom of the microcolumn, remove the rubber bulb seal, tap the column to insure that the silica gel settles uniformly. Carefully pack column until the silica gel reaches the 120 ± 2 mm mark. Be sure that there are no air bubbles in the column. Add about 10 mm of sodium sulfate to the top of the silica gel. Under low humidity conditions, the silica gel may coat the sides of the column and not settle properly. This can be minimized by wiping the outside of the column with an anti-static solution.

b Deactivation of the Silica Gel

1) Fill the microcolumn to the base of the 10/30 joint with the 0.50% ether-benzene mixture, assemble reservoir (using spring clamps) and fill with approximately 15 ml of the 0.50% ether-benzene mixture. Attach the air pressure device (using spring clamps) and adjust the elution rate to approximately 1 ml/min. with the air pressure control. Release the air pressure and detach reservoir just as the last of the solvent enters the

sodium sulfate. Fill the column with n-hexane (not mixed hexanes) to the base of the 10/30 fitting. Evaporate all residual benzene from the reservoir, assemble the reservoir section and fill with 5 ml of n-hexane. Apply air pressure and adjust the flow to 1 ml/min. (The n-hexane flows slightly faster than the benzene). Release the air pressure and remove the reservoir just as the n-hexane enters the sodium sulfate. The column is now ready for use.

2) Pipet a 1.0 ml aliquot of the concentrated sample extract (previously reduced to a total volume of 2.0 ml) on to the column. As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with 0.25 ml of n-hexane. Then assemble the upper section of the column. As the last of the n-hexane rinse reaches the surface of the sodium sulfate, add enough n-hexane (volume predetermined, see X D 3 below) to just elute all of the PCB's present in the sample. Apply air pressure and adjust until the flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see X D 3 below) in an accurately calibrated ampul. As the last of the n-hexane reaches the surface of the sodium sulfate, release the air pressure and change the collection ampul.

3) Fill the column with 0.50% diethyl ether in benzene, again apply air pressure and adjust flow to 1 ml/min. Collect the eluate until all of the organochlorine pesticides of interest have been eluted (volume predetermined, see X D3 below).

4) Analyze the eluates by gas chromatography.

3 Determination of Elution Volumes

a The elution volumes for the PCB's and the pesticides depend upon a number of factors which are difficult to control. These include variation in:

1) Mesh size of the silica gel

2) Adsorption properties of the silica gel

3) Polar contaminants present in the eluting solvent

4) Polar materials present in the sample and sample solvent

5) The dimensions of the microcolumns

Therefore, the optimum elution volume must be experimentally determined each time a factor is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and serially collect 1 ml elution volumes. Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for ether-benzene. Figure 2 shows the retention order of the various PCB components and of the pesticides. Using this information, prepare the mixtures required for calibration of the microcolumn.

b In determining the volume of hexane required to elute the PCB's the sample volume (1 ml) and the volume of n-hexane used to rinse the column wall must be considered. Thus, if it is determined that a 10.0 ml elution volume is required to elute the PCB's, the volume of hexane to be added in addition to the sample volume but including the rinse volume should be 9.5 ml.

c Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (IX D) indicates which Aroclors are present and provides the basis for selection of the ideal elution volume. This helps to minimize the quantity of organochlorine pesticides which will elute along with the low percent chlorine PCB's and insures the most efficient separations possible for accurate analysis.

d For critical analysis where the PCB's and pesticides are not separated completely, the column should be accurately calibrated according to (X D 3 a) to determine the percent of material of interest that elutes in each fraction. Then flush the column with an additional 15 ml of 0.50% ether in benzene followed by 5 ml of n-hexane and use this reconditioned column for the sample separation. Using this technique one can accurately predict the amount (%) of materials in each micro column fraction.

E Micro Column Separation of Sulfur, PCB's, and Pesticides

1 See procedure for preparation and packing micro column in PCB analysis section (X D 1 and X D 2).

2 Microcolumn Calibration

a Calibrate the microcolumn for sulfur and PCB separation by collecting 1.0 ml fractions and analyzing them by gas chromatography to determine the following:

1) The fraction with the first eluting PCB's (those present in 1260),

2) The fraction with the last eluting PCB's (those present in 1221),

3) The elution volume for sulfur,

4) The elution volume for the pesticides of interest in the 0.50% ether-benzene fraction.

From these data determine the following:

1) The eluting volume containing only sulfur (Fraction I),

2) The eluting volume containing the last of the sulfur and the early eluting PCB's (Fraction II),

3) The eluting volume containing the remaining PCB's (Fraction III),

4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV).

3 Separation Procedure

a Carefully concentrate the 6% eluate from the florisil column to 2.0 ml in the graduated ampul on a warm water bath.

b Place 1.0 ml (50%) of the concentrate into the microcolumn with a 1 ml pipet. Be careful not to get any sulfur crystals into the pipet.

c Collect Fractions I and II in calibrated centrifuge tubes.

Collect Fractions III and IV in calibrated ground glass stoppered ampules.

d Sulfur Removal(9) - Add 1 to 2 drops of mercury to Fraction II stopper and place on a wrist-action shaker. A black precipitate indicates the presence of sulfur. After approximately 20 minutes the mercury may become entirely reacted or deactivated by the precipitate. The sample should be quantitatively transferred to a clean centrifuge tube and additional mercury added. When crystals are present in the sample, three treatments may be necessary to remove all the sulfur. After all the sulfur has been removed from Fraction II (check using gas chromatography) combine Fractions II and III. Adjust the volume to 10 ml and analyze gas chromatographically. Be sure no mercury is transferred to the combined Fractions II and III, since it can react with certain pesticides.

By combining Fractions II and III, if PCB's are present, it is possible to identify the Aroclor(s) present and a quantitative analysis can be performed accordingly. Fraction I can be discarded since it only contains the bulk of the sulfur. Analyze Fractions III and IV for the PCB's and pesticides. If DDT and its homologs, aldrin, heptachlor, or technical chlordane are present along with the PCB's, an additional micro-column separation can be performed which may help to further separate the PCB's from the pesticides (See X D).

XI QUANTITATIVE DETERMINATION

A Measure the volume of n-hexane eluate, containing the PCB's and inject 1 to 5 µl into the gas chromatograph. If necessary, adjust the volume of the eluate to give linear response to the electron capture detector. The microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCB's.

B Calculations

1 When a single Aroclor is present, compare quantitative Aroclor reference standard (e.g., 1242, 1260) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Microgram/liter} = \frac{[A]\ [B]\ [V_t]}{[(V_i)\ \ (V_s)]} \times [N]$$

$$A = \frac{\text{ng of Standard Injected}}{\Sigma \text{ of Standard Peak Areas}} = \frac{\text{ng}}{\text{mm}^2}$$

$B = \Sigma$ of Sample Peak Areas = (mm^2)

V_i = Volume of sample injected (µl)

V_t = Volume of Extract (µl) from which sample is injected into gas chromatograph

V_s = Volume of water sample extracted (ml)

N = 2 when micro column used
1 when micro column not used

Peak Area = Peak height (mm x Peak Width at 1/2 height

2 For complex situations, use the calibration method described below. Small variations in components between different Aroclor batches make it necessary to obtain samples of several specific Aroclors. These reference Aroclors can be obtained from Dr. Ronald Webb, Southeast Environmental Research Laboratory, EPA, Athens, Georgia 30601. The procedure is as follows:

a Using the [illegible] column, chromatograph a known quantity of each Aroclor reference standard. Also chromatograph a sample of p, p'-DDE. Suggested concentration of each standard is 0.1 ng/µl for the Aroclors and 0.02 ng/µl for the p, p'-DDE.

b Determine the relative retention time (RRT) of each PCB peak in the resulting chromatograms using p, p'-DDE as 100. See Figures 3 through 5.

$$\mathrm{RRT} = \frac{\mathrm{RT} \times 100}{\mathrm{RT}_{\mathrm{DDE}}}$$

RRT = Relative Retention Time

RT = Retention time of peak of interest

$\mathrm{RT}_{\mathrm{DDE}}$ = Retention time of p, p'-DDE

Retention time is measured as that distance in mm between the first appearance of the solvent peak and the maximum for the compound.

c To calibrate the instrument for each PCB measure the area of each peak.

Area = Peak height (mm) x Peak width at 1/2 height. Using Tables 1 through 6 obtain the proper mean weight factor, then determine the response factor $\mathrm{ng/mm}^2$.

$$\mathrm{ng/mm}^2 = \frac{(\mathrm{ng}_i)\ \dfrac{(\text{mean weight percent})}{100}}{(\text{Area})}$$

ng_i = ng of Aroclor Standard Injected

Mean weight percent = obtained from Tables 1 through 6.

d Calculate the RRT value and the area for each PCB peak in the sample chromatogram. Compare the sample chromatogram to those obtained for each reference Aroclor standard. If it is apparent that the PCB peaks present are due to only one Aroclor then calculate the concentration of each PCB using the following formula:

$$\mathrm{ng\ PCB} = \mathrm{ng/mm}^2 \times \mathrm{Area}$$

Where Area = Area (mm^2) of sample peak $\mathrm{ng/mm}^2$ = Response factor for that peak measured. Then add the nanograms of PCB's present in the injection to get the total number of nanograms of PCB's present. Use the following formula to calculate the concentration of PCB's in the sample:

$$\mathrm{Micrograms/Liter} = \frac{[\mathrm{ng}]}{[V_s]}\ \frac{[V_t]}{[V_i]} \times [N]$$

V_s = volume of water extracted (ml)

V_t = volume of extract (µl)

V_i = volume of sample injected (µl)

Σng = sum of all the PCB's in nanograms for that Aroclor identified

N = 2 when microcolumn used

N = 1 when microcolumn not used

The value can then be reported as Micrograms/Liter PCB's reported as the Aroclor. For samples containing more than one Aroclor, use Figure 9 chromatogram divisional flow chart to assign a proper response factor to each peak and also identify the "most likely" Aroclors present. Calculate the ng of each PCB isomer present and sum them according to the divisional flow chart. Using the formula above, calculate the concentration of the various Aroclors present in the sample.

XII REPORTING RESULTS

A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

Table 1

Composition of Aroclor 1221(8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	31.8	15.8	1
14	19.3	9.1	1
16	10.1	9.7	2
19	2.8	9.7	2
21	20.8	9.3	2
28	5.4	13.9	2] 85% 3] 15%
32	1.4	30.1	2] 10% 3] 90%
[37 [40	1.7	48.8	3 3
Total	93.3		

[a] Retention time relative to p, p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of seventeen results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 2

Composition of Aroclor 1232 (8)

RRT[2]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	16.2	3.4	1
14	9.9	2.5	1
16	7.1	6.8	2
[20 21]	17.8	2.4	2 2
28	9.6	3.4	2] 40% 3] 60%
32	3.9	4.7	3
37	6.8	2.5	3
40	6.4	2.7	3
47	4.2	4.1	4
54	3.4	3.4	3] 33% 4] 67%
58	2.6	3.7	4
70	4.6	3.1	4] 90% 5] 10%
78	1.7	7.5	4
Total	94.2		

[a] Retention time relative to p, p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of four results as a mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 3

Composition of Aroclor 1242[(8)]

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	1.1	35.7	1
16	2.9	4.2	2
21	11.3	3.0	2
28	11.0	5.0	2] 25% 3] 75%
32	6.1	4.7	3
37	11.5	5.7	3
40	11.1	6.2	3
47	8.8	4.3	4
54	6.8	2.9	3] 33% 4] 67%
58	5.6	3.3	4
70	10.3	2.8	4] 90% 5] 10%
78	3.6	4.2	4
84	2.7	9.7	5
98	1.5	9.4	5
104	2.3	16.4	5
125	1.6	20.4	5] 85% 6] 15%
146	1.0	19.9	5] 75% 6] 25%
Total	98.5		

[a] Retention time relative to p,p'-DDE= 100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine number are bracketed.

Table 4

Composition of Aroclor 1248 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
21	1.2	23.9	2
28	5.2	3.3	3
32	3.2	3.8	3
37	8.3	3.6	3
40	8.3	3.9	3] 85% 4] 15%
47	15.6	1.1	4
54	9.7	6.0	3] 10% 4] 90%
58	9.3	5.8	4
70	19.0	1.4	4] 80% 5] 20%
78	6.6	2.7	4
84	4.9	2.6	5
98	3.2	3.2	5
104	3.3	3.6	4] 10% 5] 90%
112	1.2	6.6	5
125	2.6	5.9	5] 90% 6] 10%
146	1.5	10.0	5] 85% 6] 15%
Total	103.1		

[a] Retention time relative to p, p'-DDE= 100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 5

Composition of Aroclor 1254 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
47	6.2	3.7	4
54	2.9	2.6	4
58	1.4	2.8	4
70	13.2	2.7	4] 25% 5] 75%
84	17.3	1.9	5
98	7.5	5.3	5
104	13.6	3.8	5
125	15.0	2.4	5] 70% 6] 30%
146	10.4	2.7	5] 30% 6] 70%
160	1.3	8.4	6
174	8.4	5.5	6
203	1.8	18.6	6
232	1.0	26.1	7
Total	100.0		

[a] Retention time relative to p, p'-DDE= 100. Measured from first appearance of solvent.

[b] Standard deviation of six results as a percentage of the mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers are bracketed.

Table 6

Composition of Aroclor 1260(8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
70	2.7	6.3	5
84	4.7	1.6	5
[98 104	3.8	3.5	5 60% 6 40% [d]
117	3.3	6.7	6
125	12.3	3.3	5 15% 6 85%
146	14.1	3.6	6
160	4.9	2.2	6 50% 7 50%
174	12.4	2.7	6
203	9.3	4.0	6 10% 7 90%
[232 244	9.8	3.4	6 10% 8 90% [e]
280	11.0	2.4	7
332	4.2	5.0	7
372	4.0	8.6	8
448	.6	25.3	8
528	1.5	10.2	8
Total	98.6		

[a] Retention time relative to p, p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b] Standard deviation of six results as a mean of the results.

[c] From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[d] Composition determined at the center of peak 104.

[e] Composition determined at the center of peak 232.

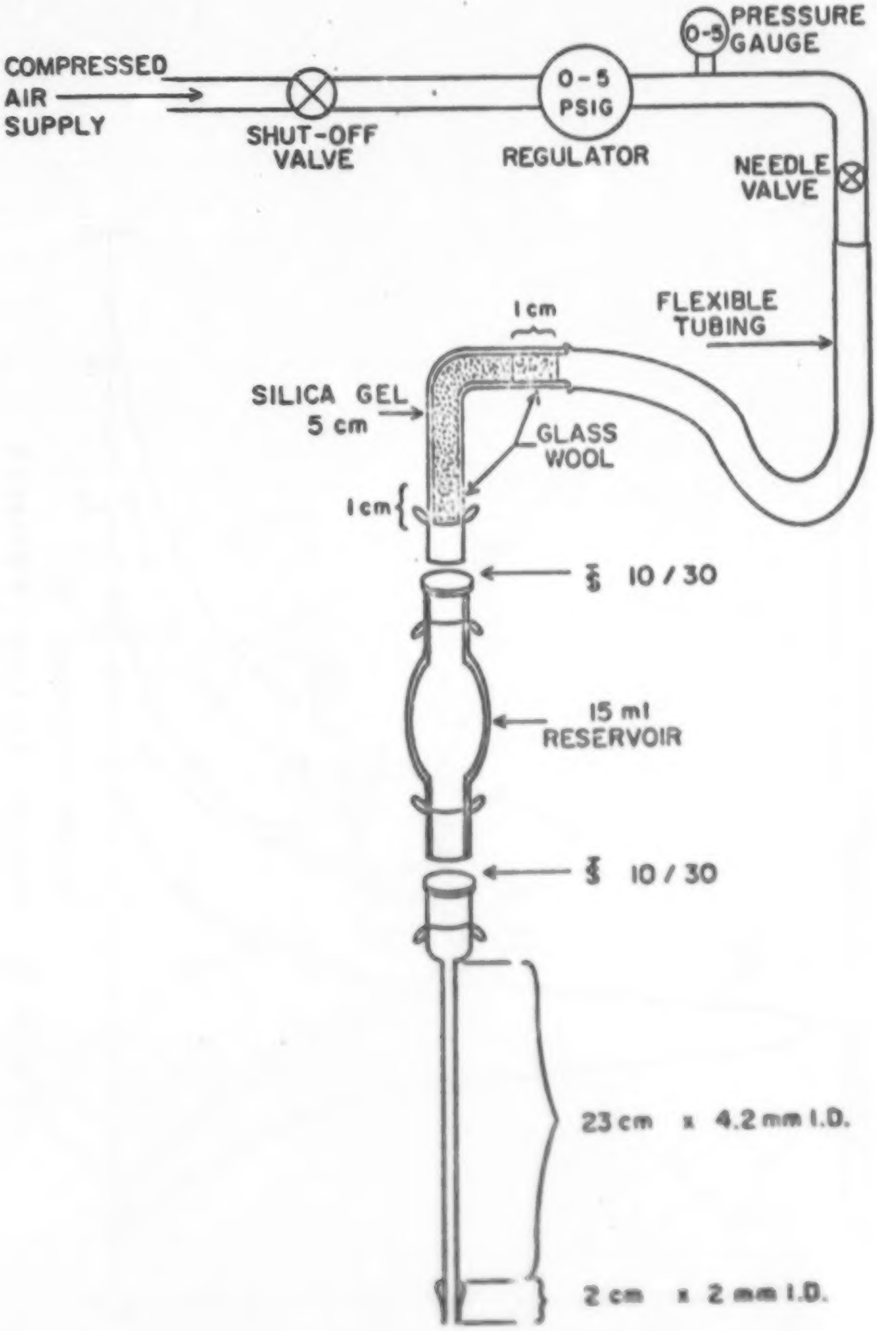
COMPRESSED
AIR
SUPPLY
SHUT-OFF VALVE
0-5 PSIG
REGULATOR
0-5
PRESSURE GAUGE
NEEDLE VALVE
FLEXIBLE TUBING
1 cm
SILICA GEL 5 cm
GLASS WOOL
1 cm
$ 10 / 30
15 ml RESERVOIR
$ 10 / 30
23 cm x 4.2 mm I.D.
2 cm x 2 mm I.D.

FIGURE 1. MICROCOLUMN SYSTEM

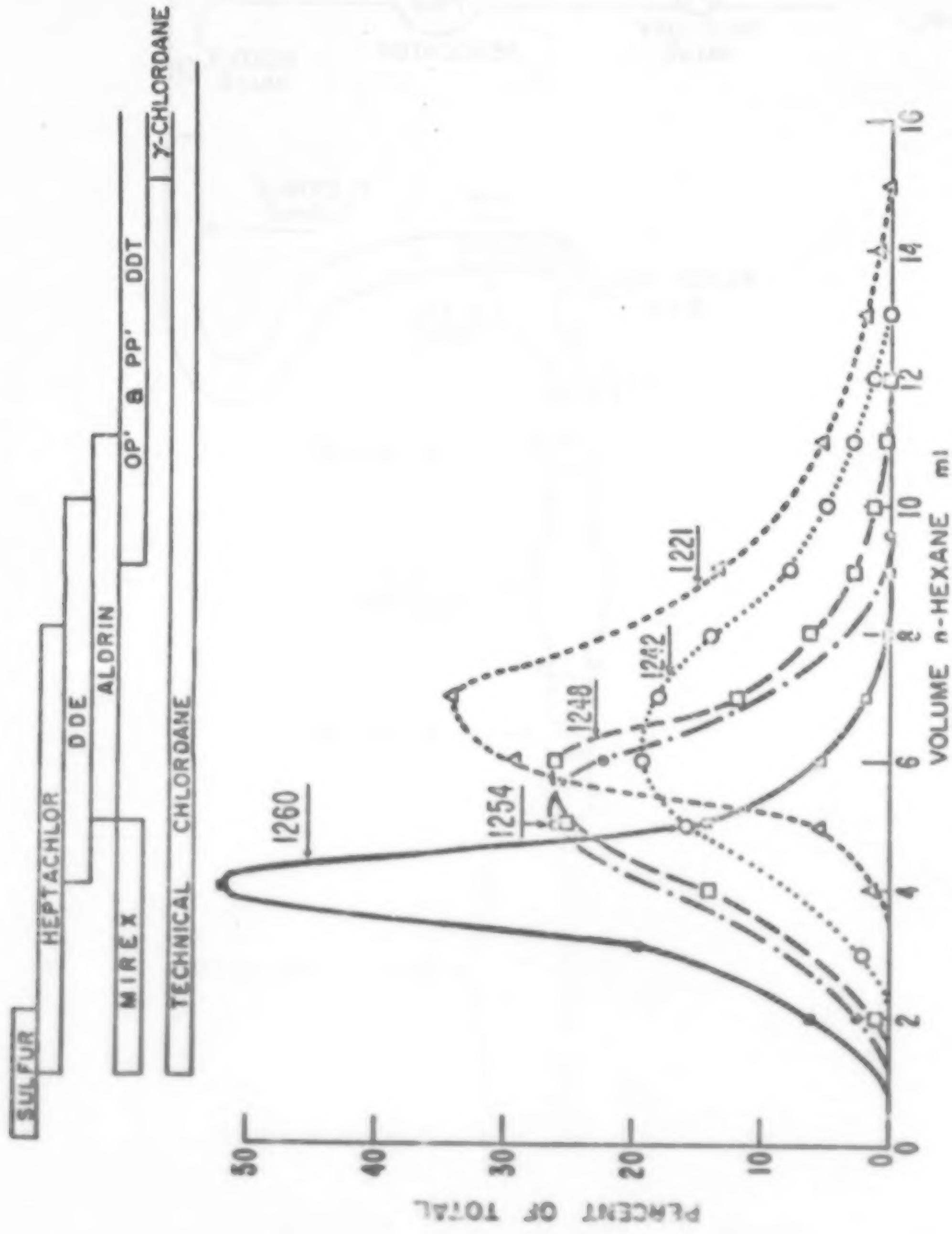

Figure 2. Aroclor Elution Patterns

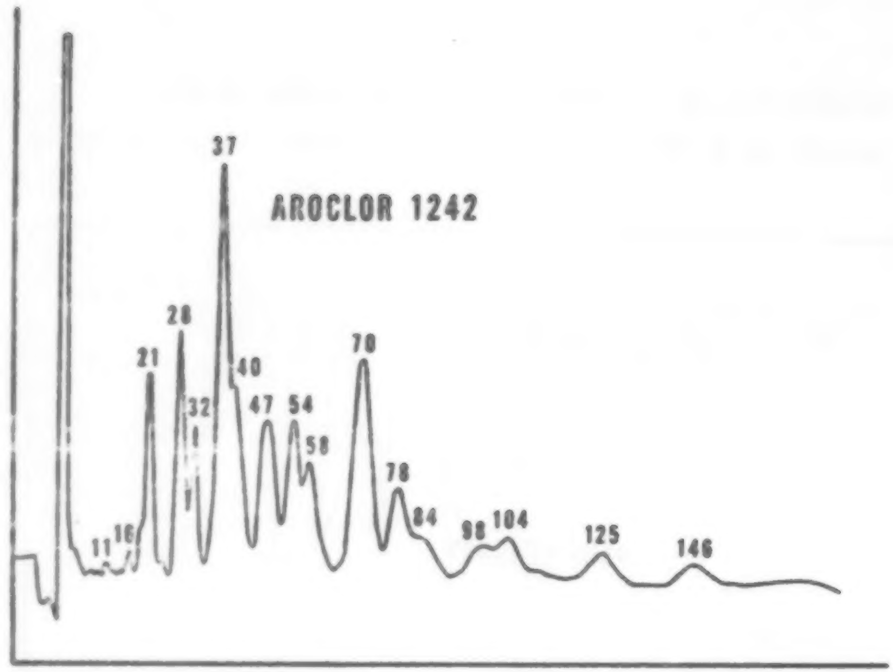

Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture

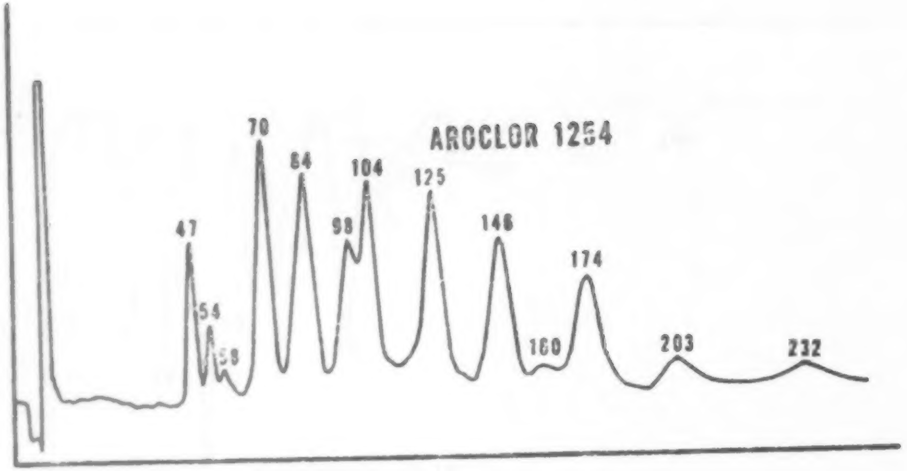

Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

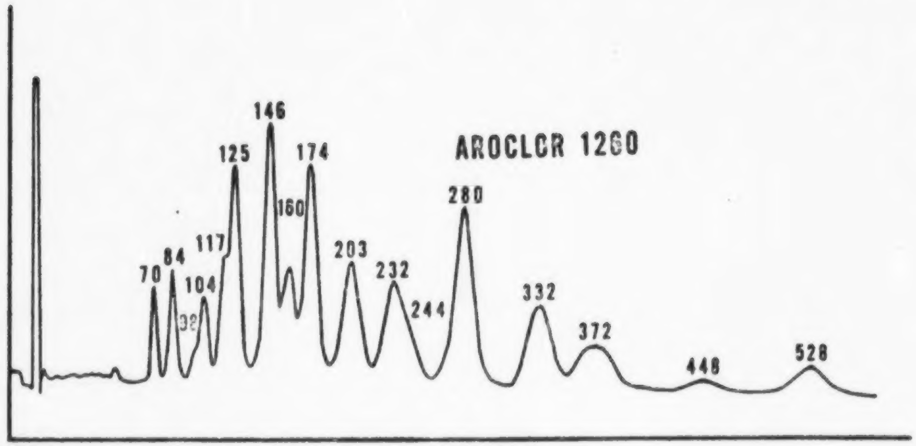

Figure 5. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

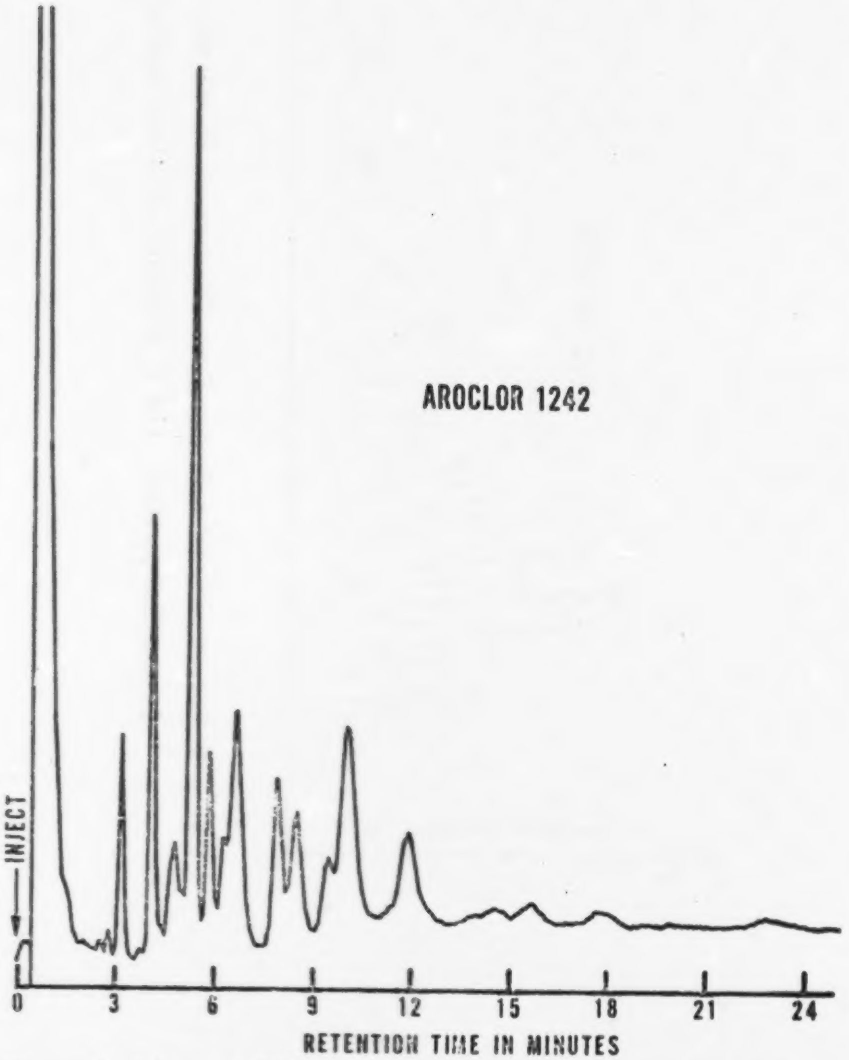

Figure 6. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

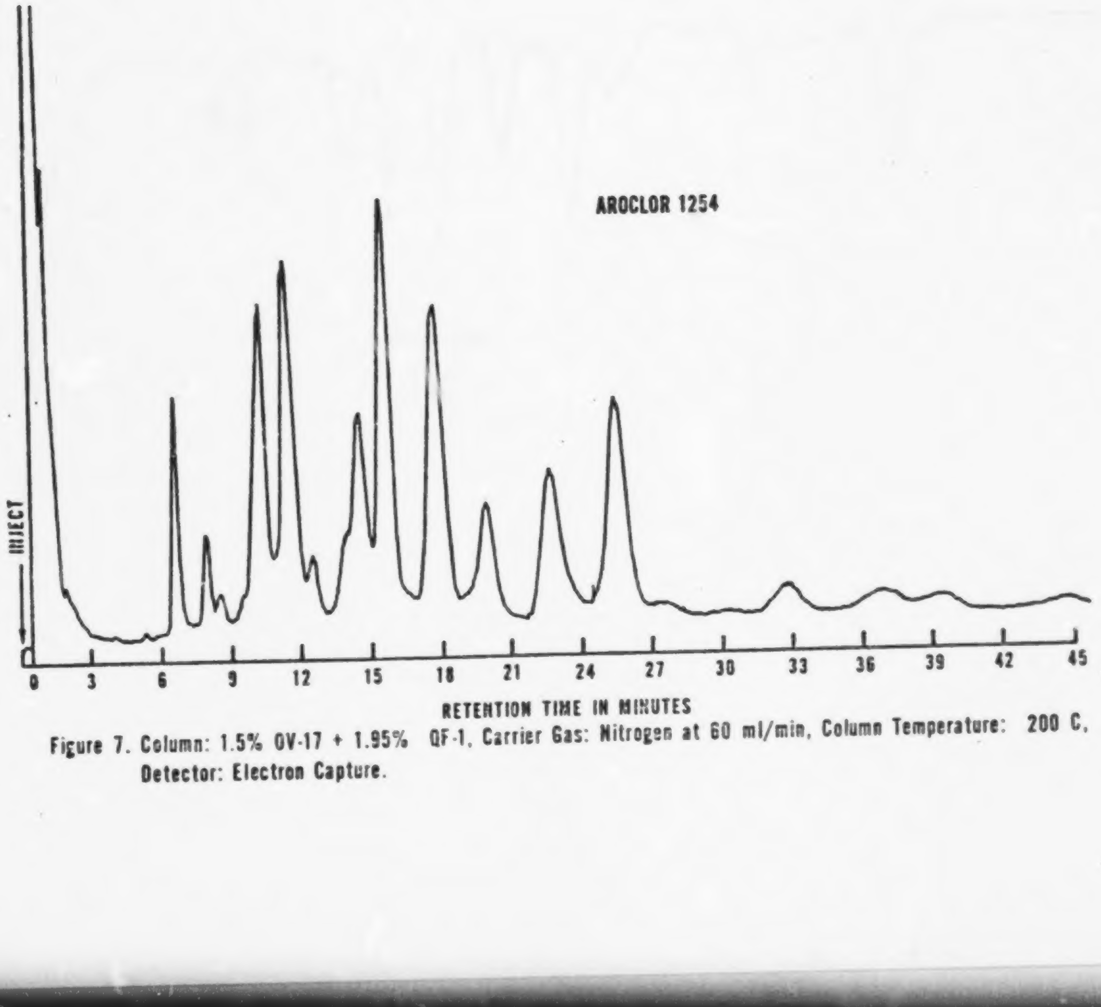

Figure 7. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

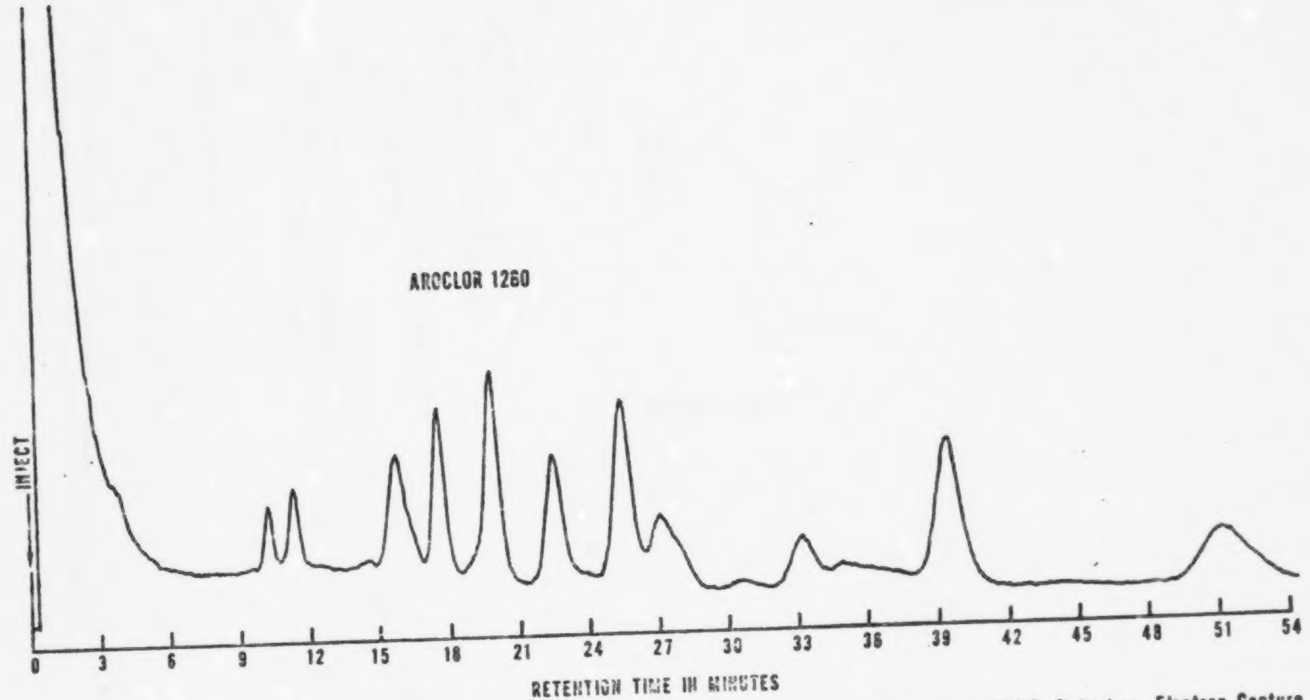

Figure 8. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200C, Detector: Electron Capture.

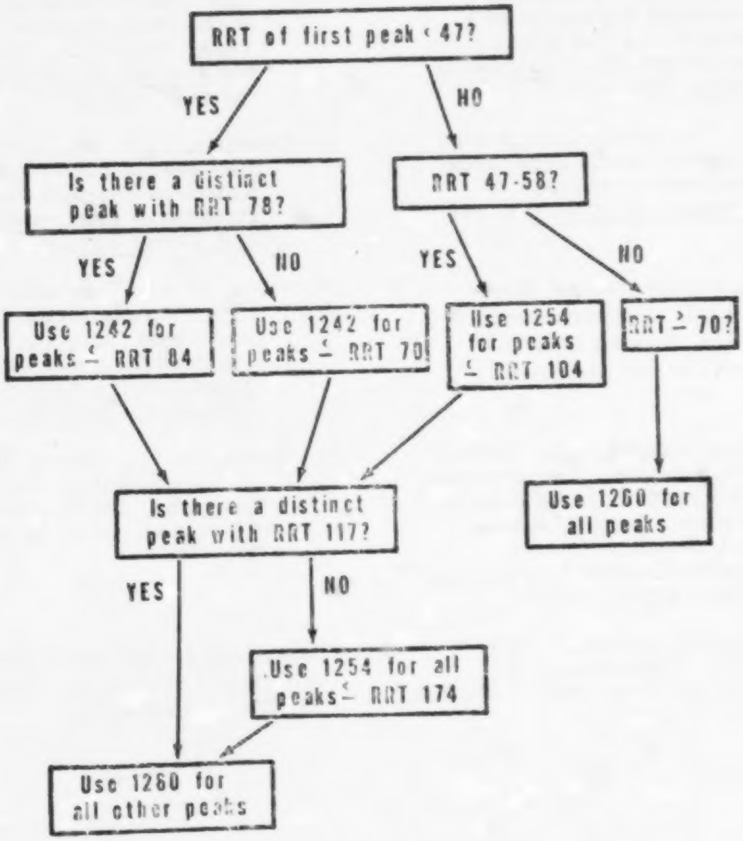
RRT of first peak < 47?
YES
NO
Is there a distinct peak with RRT 78?
RRT 47-58?
YES
NO
YES
NO
Use 1242 for peaks ≤ RRT 84
Use 1242 for peaks ≤ RRT 70
Use 1254 for peaks ≤ RRT 104
RRT ≥ 70?
Is there a distinct peak with RRT 117?
Use 1260 for all peaks
YES
NO
Use 1254 for all peaks ≤ RRT 174
Use 1260 for all other peaks

Figure 9. Chromatogram Division Flowchart (8).

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Descriptors: Chemical Analysis, Polychlorinated Biphenyls, Chromatography, Gas Chromatography

APPENDIX I

XIII STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6), (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

1 Buret. – 25 ml with 1/10 ml graduations.

2 Erlenmeyer flasks. – 125 ml narrow mouth and 25 ml, glass stoppered.

3 Pipet. – 10 and 20 ml transfer.

4 Volumetric flasks. – 500 ml.

C Reagents and Solvents

1 Alcohol, ethyl. – USP or absolute, neutralized to phenolphthalein.

2 Hexane. – Distilled from all glass apparatus.

3 Lauric acid. – Purified, CP.

4 Lauric acid solution. – Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

5 Phenolphthalein Indicator. – Dissolve 1 g in alcohol and dilute to 100 ml.

6 Sodium hydroxide. – Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

METHOD FOR ORGANOCHLORINE PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

A This method covers the determination of various organochlorine pesticides, including some pesticidal degradation products and related compounds in industrial effluents. Such compounds are composed of carbon, hydrogen, and chlorine, but may also contain oxygen, sulfur, phosphorus, nitrogen or other halogens.

B The following compounds may be determined individually by this method with a sensitivity of 1 μg/liter: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, pentachloronitrobenzene and trifluralin. Under favorable circumstances, Strobane, toxaphene, chlordane (tech.) and others may also be determined. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

C When organochlorine pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

II SUMMARY

A The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and/or the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography and liquid-liquid partition, methods for elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography. Results are reported in micrograms per liter.

B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the anlaysis. Specific selection of reagents and purification solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4 and 1.5.(1)

B The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organochlorine pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organochlorine pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

C Polychlorinated Biphenyls (PCB's) - Special attention is called to industrial plasticizers and hydraulic fluids such as the PCB's which are a potential source of interference in pesticide analysis. The presence of PCB's is indicated by a large number of partially resolved or unresolved peaks which may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures.(2, 3)

D Phthalate Esters - These compounds, widely used as plasticizers, respond to the electron capture detector and are a source of interference in the determination of organochlorine pesticides using this detector. Water leaches these materials from plastics, such as polyethylene bottles and tygon tubing. The presence of phthalate esters is implicated in samples that respond to electron capture but not to the microcoulometric or electrolytic conductivity halogen detectors or to the flame photometric detector.

E Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, e.g., parathion, also respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. Such compounds can be identified by their response to the flame photometric detector.(4)

IV APPARATUS AND MATERIALS

A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

1 Electron Capture - Radioactive (tritium or nickel 63)

2 Microcoulometric Titration

3 Electrolytic Conductivity

C Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

D Gas Chromatographic Column Materials:

1 Tubing - Pyrex (180 cm long x 4 mm ID)

2 Glass Wool - Silanized

3 Solid Support - Gas-Chrom Q (100-120 mesh)

4 Liquid Phases - Expressed as weight percent coated on solid support.

a OV-1, 3%

b OV-210, 5%

c OV-17, 1.5% plus QF-1, 1.95%

d QF-1, 6% plus SE-30, 4%

E Kuderna-Danish (K-D) Glassware (Kontes)

1 Snyder Column - three ball (macro) and two ball (micro)

2 Evaporative Flasks - 500 ml

3 Receiver Ampuls - 10 ml, graduated

4 Ampul Stoppers

F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50 and 100 μl

I Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

J Blender - High speed, glass or stainless steel cup.

K Graduated cylinders - 100, 250 and 1000 ml.

L Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

V REAGENTS, SOLVENTS, AND STANDARDS

A Ferrous Sulfate - (ACS) 30% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

D Sodium Hydroxide - (ACS) 10 N in distilled water.

E Sodium Sulfate - (ASC) Granular, anhydrous (conditioned @ 400 C for 4 hours).

F Sulfuric Acid - (ASC) Mix equal volumes of concentration H_2SO_4 with distilled water.

G Diethyl Ether - Nanograde, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

3 Distill deperoxidized ether in glass and add 2% ethanol.

H Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - nanograde, redistill in glass if necessary.

I Pesticide Standards - Reference grade.*

VI CALIBRATION

A Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when $\leq$ 0.06 ng is injected for electron capture detection and $\leq$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

B Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.

C The elution order and retention ratios of various organochlorine pesticides are provided in Table 1, as a guide.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.(5)

*May be ordered from US EPA, EMSL, Quality Assurance Branch, Cincinnati, Ohio 45268.

B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

VIII SAMPLE PREPARATION

A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

B For a sensitivity requirement of μg/l, when using microcoulometric or electrolytic conductivity methods for detection, 100 ml or more of sample will be required for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

C Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

IX EXTRACTION

A Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

B Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. After prewetting 3-4 inches of anhydrous sodium sulfate in a column, pour the organic layer into a 100 ml beaker, pass it through the column, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

C Concentrate the extract in the K-D evaporator on a hot water bath.

D Analyze by gas chromatography unless a need for cleanup is indicated (See Section X).

X CLEAN-UP AND SEPARATION PROCEDURES

A Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

B Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides. Of the pesticides listed in Scope I B only mirex is not efficiently recovered.

1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with

two 100 ml portions of hexane by vigorously shaking about 15 seconds.

3 Combine the hexane extract in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

5 Analyze by gas chromatography unless a need for further cleanup is indicated.

C Florisil Column Adsoprtion Chromatography

1 Adjust the sample extract volume to 10 ml.

2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition D).

Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

5 Analyze by gas chromatography.

D Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin
BHC
Chlordane
DDD
DDE
DDT
Heptachlor
Heptachlor Expoxide
Lindane
Methoxychlor
Mirex
Pentachloronitrobenzene
Strobane
Toxaphene
Trifluralin
PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual.(6)

XI CALCULATION OF RESULTS

A Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2.(1)

(1) $$\text{Micrograms/liter} = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

$$A = \frac{\text{ng standard}}{\text{Standard area}}$$

B = Sample aliquot area

V_i = Volume of extract injected (μl)

V_t = Volume of total extract (μl)

V_s = Volume of water extracted (ml)

XII REPORTING RESULTS

A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

Table 1

RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200 C	180 C	180 C	200 C
Argon/Methane Carrier Flow	60 ml/min	70 ml/min	70 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Trifluralin	0.39	1.11	0.33	0.57
α-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p'-DDT	3.16	2.70	2.69	2.39
p,p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

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[1] All columns glass, 180 cm x 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)

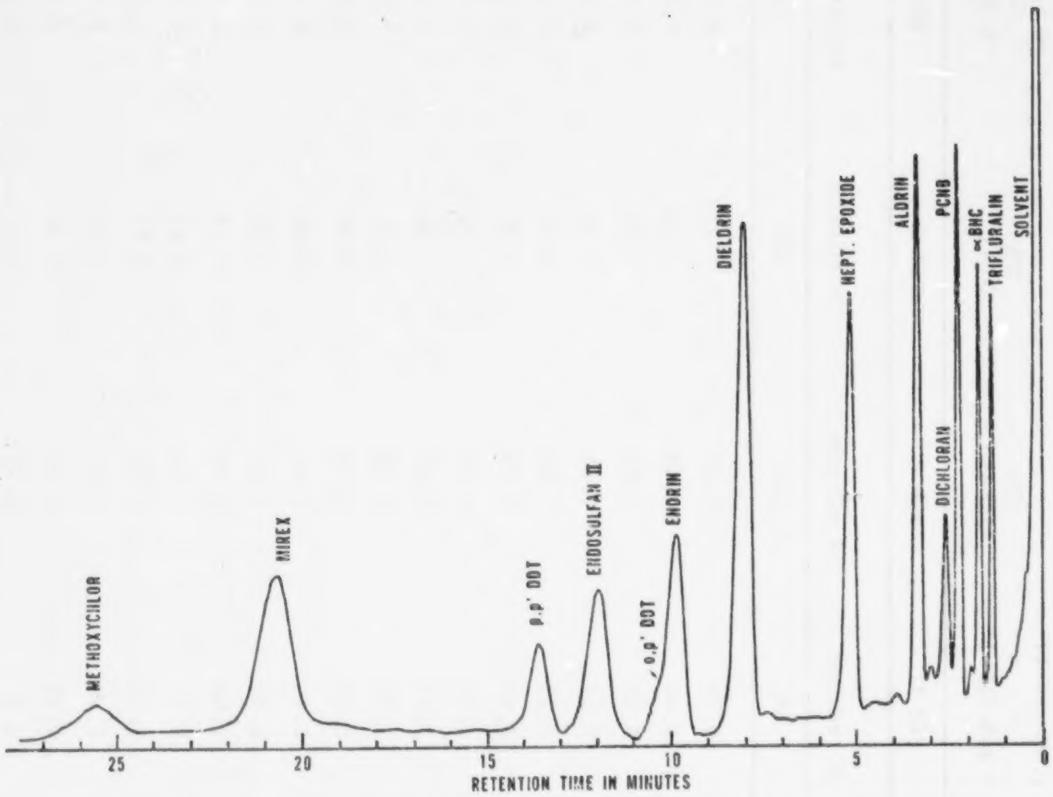

Figure 1. Column Packing: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

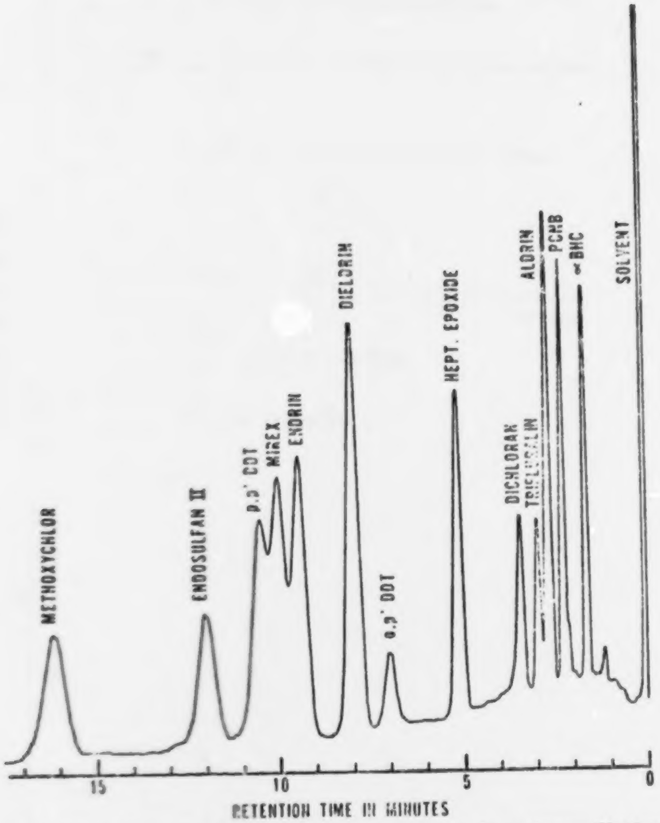

Figure 2. Column Packing: 5% OV-210, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

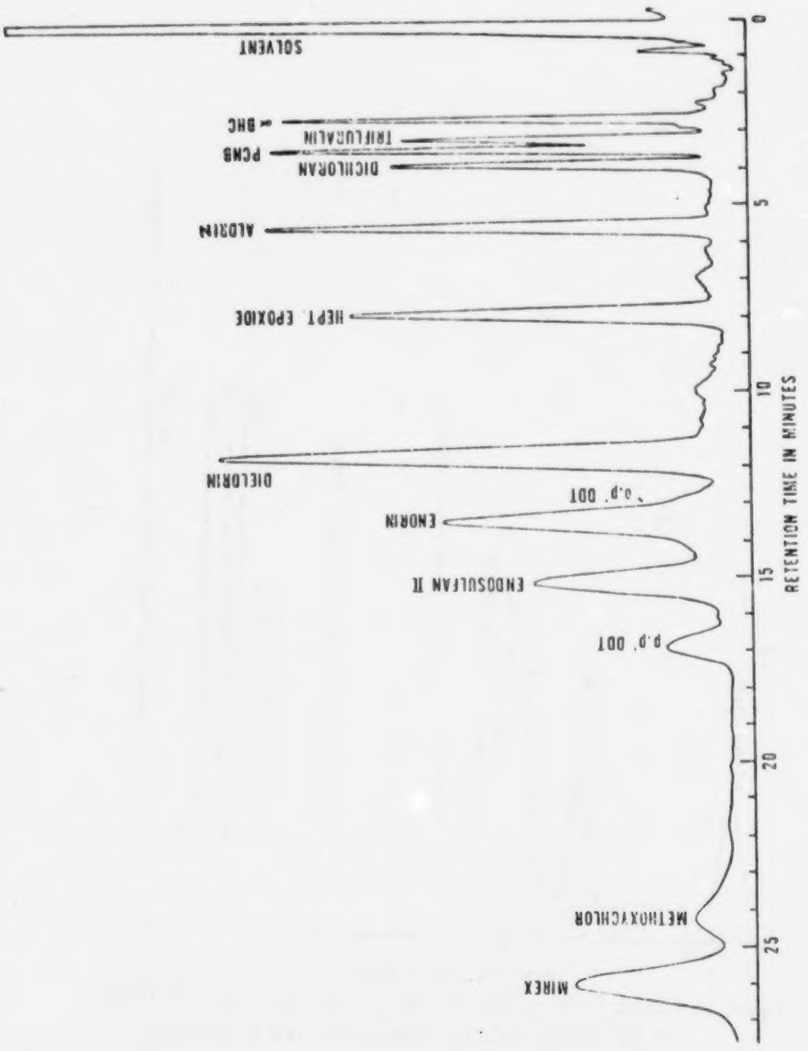

Figure 3. Column Packing: 6% QF-1 + 4% SE-30. Carrier Gas: Argon/Methane at 60 ml/min. Column Temperature: 200 C. Detector: Electron Capture.

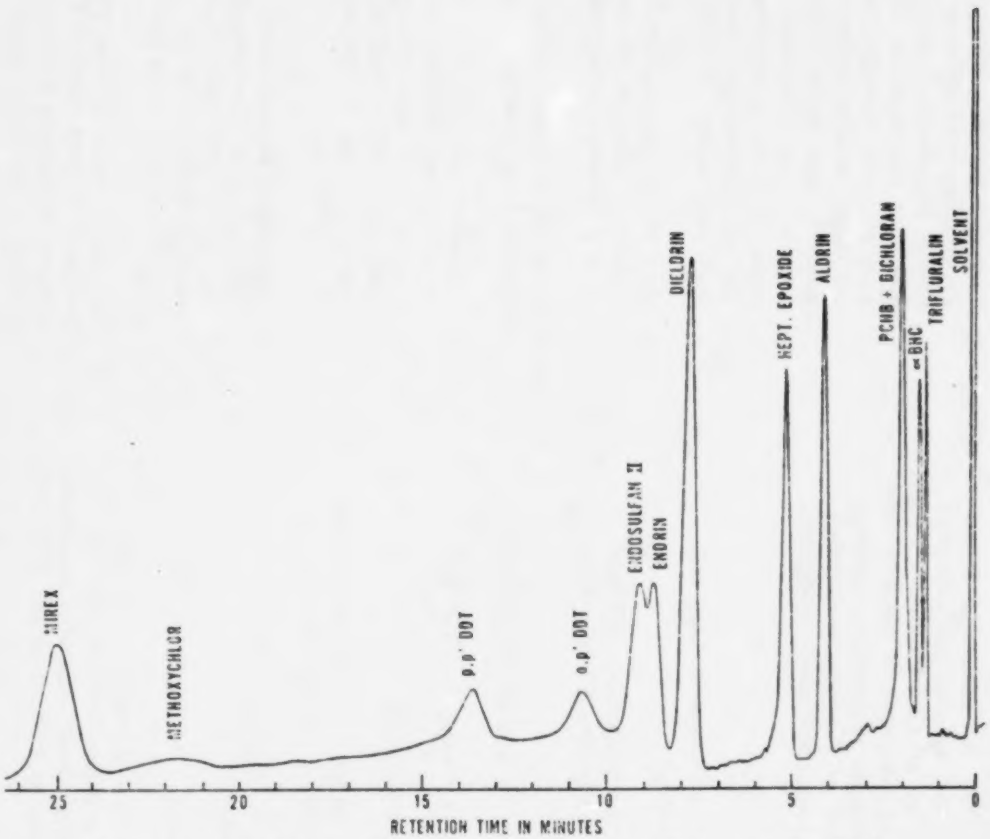

Figure 4. Column Packing: 3% OV-1, Carrier Gas: Argon/Methane at 70 ml/min. Column Temperature: 180 C, Detector: Electron Capture.

APPENDIX I

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3 Pipet. – 10 and 20 ml transfer.

4 Volumetric flasks. – 500 ml.

C Reagents and Solvents

1 Alcohol, ethyl. – USP or absolute, neutralized to phenolphthalein.

2 Hexane. – Distilled from all glass apparatus.

3 Lauric acid. – Purified, CP.

4 Lauric acid solution. – Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

5 Phenolphthalein Indicator. – Dissolve 1 g in alcohol and dilute to 100 ml.

6 Sodium hydroxide. – Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeryer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

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1 Calculate amount of lauric acid adsorbed on Florisil as follows: Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

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Descriptors: Organic Compounds Pesticides, Chlorinated Hydrocarbon Pesticides, Techniqes Analytical, Chromatography, Gas Chromatography